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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6: <b>A61K 38/00, 45/00, C07K 14/74</b>		A1	(11) International Publication Number: <b>WO 95/05189</b> (43) International Publication Date: <b>23 February 1995 (23.02.95)</b>
(21) International Application Number: <b>PCT/US94/09189</b> (22) International Filing Date: <b>12 August 1994 (12.08.94)</b>		(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  Published <i>With international search report. With amended claims.</i>	
(30) Priority Data: <b>08/105,416 12 August 1993 (12.08.93) US</b>			
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(54) Title: CLASS I MHC MODULATION OF SURFACE RECEPTOR ACTIVITY			
(57) Abstract			
Methods and compositions are provided for regulating surface membrane receptor responses by modulating interaction between MHC Class I antigen and the surface membrane receptor. Various techniques may be employed for enhancing or reducing the interaction between the MHC Class I antigen and surface membrane receptor (e.g., enhancing surface expression of the MHC Class I antigen or employing agents which affect interaction between MHC Class I antigen and surface receptors). The aggregative characteristics of oligopeptides which act as agents in affecting interaction between MHC Class I antigen and surface receptors may be employed in a screening assay for determining drugs which affect interaction between Class I antigen and surface receptors. Active peptide aggregative characteristics may also be employed in a method of administration of effectors of surface receptor response modulation.			

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**CLASS I MHC MODULATION OF SURFACE  
RECEPTOR ACTIVITY**

**INTRODUCTION**

This application is a continuation-in-part of application Serial No. 08/057,184 filed May 3, 1993, which is a continuation of application Serial No. 07/649,471 filed February 1, 1991, now abandoned, which is a continuation-in-  
5 part of application Serial No. 07/323,565 filed March 14, 1989, which is a continuation-in-part of application Serial No. 07/028,241 filed March 20, 1987, now abandoned.

**Technical Field**

The field of the subject invention concerns modulation  
10 of surface membrane receptor responses.

**Background**

The major histocompatibility complex (MHC) Class I antigens are expressed on virtually all types of vertebrate cells examined. These highly polymorphic transmembrane  
15 glycoproteins have a 45 kD heavy chain consisting of a short cytoplasmic C-terminal tail, a transmembrane region, and an extracellular N-terminal sequence which encompasses three domains ( $\alpha_1$ ,  $\alpha_2$ , and  $\alpha_3$ ).  $\alpha_1$ - and  $\alpha_2$ -domains carry all the immunological polymorphism, while the membrane-proximal  $\alpha_3$   
20 domain is non-covalently associated with the 12 kD  $\beta_2$  microglobulin.

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The MHC Class I antigen plays an essential role in restriction of the target cell repertoire of cytotoxic T-lymphocytes (CTL). Restriction involves preferential utilization of the different polymorphic MHC Class I antigens, H-2K, -D or -L (for mouse) or HLA-A, -B, or -C (for human), e.g. in recognition of virally infected cells. For the most part, attention has been directed to the role of the MHC Class I antigens in restricting T-cell activity. However, in addition to restriction of the immune response, Class I MHC antigens may also interact with receptors expressed on the cell surface.

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Relevant Literature

For a review of biological functions of MHC Class I antigens see Ohno, Immunol. Rev. (1977) 33:59-69; and Simonsen, Prog. Allergy (1985) 36:151-176. For a description 5 of the insulin receptor see Cuatrecasas, J. Biol. Chem. (1972) 247:1980-1991; Kasuga et al., ibid. (1983) 258:10392-10399; and Kasuga et al., ibid. (1983) 258:10973-10980. For suggestion that Class I antigens and insulin receptors interact, see Olsson, In Cell Fusion: Gene Transfer and 10 Transformation (eds. Beers & Bassett) 395-403 (Raven Press, New York, 1984); Simonsen and Olsson, Ann. Immunol. (1983) 134D:85-92; and Stagsted, et al. Cell (1990) 62:297-307. Other evidence supporting the interaction between MHC products and insulin receptor may be found in Fehlman et 15 al., Phillips et al., ibid. (1986) 83:3474-3478; Due et al., ibid. (1986) 83:6007-6001, and Samson et al., J. Immunology (1986) 137:2293-2298. Evidence for the inhibition of insulin receptor phosphorylation by MHC Class I peptides can be found in Hansen, et al. Proc. Natl. Acad. Sci. USA (1989) 86:3123-3126. Suggestions of a correlation 20 between over-expression of certain Class I products and increased metastatic potential of particular tumors may be found in Wallich et al., Nature (1985) 315:301-305; Katzav et al., Int. J. Cancer (1984) 33:47-415; Olsson, Cancer Rev. 25 (1986) 3:91-114; and Goodnow et al., Science (1985) 230:777-783.

A structural association between epidermal growth factor receptor (EGFR) and MHC Class I on human cells has been suggested by Schreiber et al., J. Cell Biol. (1984) 30 98:725-731 and Phillips et al., Proc. Natl. Acad. Sci. USA (1986) 83:3474-3478. The effect of MHC Class I peptides on glucose transport mediated by EGFR can be found in Stagsted, et al. J. Biol. Chem. (1993) 268(3):1770-1774. A review of EGFR and its functions may be found in Carpenter and Cohen, 35 Ann. Rev. Biochem. (1979) 48:193-216 and Carpenter, J. Cell Sci. Suppl. (1985) 3:1-9.

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Other receptors of interest and appropriate references of relevance to these receptors include: IGF-I, Rosen Science (1987) 237:1452-1458; IGF-II, Simpson and Cushman Ann. Rev. Biochem. (1986) 55:1059-1089; LDL receptor, Hobbs, 5 et al. Human Mutat. (1992) 1:445-466; scavenger LDL receptor, Krieger, et al. J. Biol. Chem. (1993) 268:4569-4572; and  $\beta_2$ -adrenergic receptor, Pitcher, et al. Science (1992) 257:1264-1267.

SUMMARY OF THE INVENTION

10 Methods and compositions are provided for modulating the activity of cell surface receptors. The methods of the subject invention may employ up or down regulation of MHC Class I antigens to modulate surface expression of receptors. Alternatively, the methods of the subject 15 invention may modulate surface expression of surface receptors by employing substances which are agonistic or antagonistic to the interactions between MHC Class I antigen, surface receptors or other molecules which mediate surface receptor internalization. The methods and 20 compositions of the subject invention may be used in diagnosis and therapy of diseases which involve inadequate or inappropriate receptor response as well as in the screening of drug candidates which may affect surface expression of receptors.

25 BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Summary of relevance of each residue in D<sup>k</sup>-(69-85) for biological activity, (insulin-stimulated glucose transport in rat adipose cells) and ordered structure in the MHC Class I peptides. In panel A, open circles indicate 30 residues where substitution with alanine has no effect on activity; shaded circles indicate residues, which when substituted with alanine, result in reduced activity; solid circles indicate residues, which when substituted with alanine, result in complete loss of activity; ^, ~ and =

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indicate residues, which when simultaneously substituted with alanine, result in complete loss of activity; and \* indicates a residue, which when substituted with alanine, results in loss of ordered structure and therefore complete  
5 loss of activity. Panel B depicts the helical rod of the peptide. Symbols encircled by a solid line or a dotted line indicate that the residue is localized on the front or back of the rod, respectively. Panel C depicts the helical rod of the peptide as viewed on end.

10 Figure 2. Effect of D<sup>k</sup>-(62-85) on IGF-II cross-linking and binding to rat adipose cells. Isotherm and competition binding curves of <sup>125</sup>I-IGF-II ligand in the presence (solid circles) or absence (open circles) of 10 mM D<sup>k</sup>-(62-85) are presented in panels A and B, respectively.

15 Figure 3. Effects of [Ala<sup>85</sup>]-D<sup>k</sup>-(69-85) on the kinetics of GLUT4 internalization in rat adipose cells.

Figure 4. Effects of [Ala<sup>85</sup>]-D<sup>k</sup>-(69-85) on the kinetics of the IGF-II receptor internalization in rat adipose cells.

20 Figure 5. Internalization of bound <sup>125</sup>I-IGF-I to chinese hamster ovary cells as a function of increasing D<sup>k</sup>-(69-85) peptide concentration.

Figure 6. Effect of D<sup>k</sup>-(62-85) peptide on binding of labeled transferrin to rat adipose cells.

25 Figure 7. Effect of [Ala<sup>85</sup>]-D<sup>k</sup>-(69-85) peptide on the subcellular localization of low-density lipoprotein receptor in human fibroblasts.

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DESCRIPTION OF SPECIFIC EMBODIMENTS

Methods and compositions are provided for modulating the response of cell surface receptors by affecting the interaction between the cell surface receptor and major histocompatibility (MHC) Class I antigen which in turn affects the surface expression of the receptor. Surface expression of receptors as used herein is intended to mean the appearance of the receptors at the cell surface. Thus, an increase in surface expression of a receptor results in an increase in the population of receptors on the cell surface. Modulation of surface expression of receptors, and thus the biological activity of the receptors, may be achieved through up or down regulation of Class I antigen surface expression or surface translocation. Alternatively, surface expression of receptors may be achieved by providing agents which may act as agonists or antagonists affecting the ability of Class I MHC to mediate receptor internalization or the susceptibility of the receptor to such internalization mediated by Class I MHC antigen.

Agents which may act as such agonists or antagonists may include oligopeptides, antibodies and the like. As the precise mechanism whereby MHC Class I antigens and agonistic or antagonistic agents modulate surface receptors is unknown, reference to interactions between MHC antigens, agents and receptors are intended to include direct binding between any of these molecules as well as indirect interactions, such as those mediated through other potential, though yet unidentified, accessory molecules. Modulation of the Class I antigen-receptor interaction can be used in diagnosing and treating a large variety of conditions associated with cellular membrane receptors.

MHC Class I antigens include human MHC Class I antigens and mammalian equivalents thereof, such as Class I antigens of the H-2 locus of mice, in particular H-2 D and K. Human MHC Class I antigens include, for example, HLA-A, B, C, Qa and T1. Of particular interest in the modulation of

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cellular receptors are the  $\alpha_1$ - and  $\alpha_2$ -domains of MHC Class I antigens, more particularly the  $\alpha_1$ -domain. Of more particular interest are the amino acid sequences involved in the polymorphic regions of  $\alpha_1$ - and  $\alpha_2$ -, ranging from amino acid 50 to amino acid 90, more particularly amino acids 55 to 90, usually 60 to 90, more particularly 62 to 90 or 90 to 120; more usually 90 to 116, where the amino acid sequences of interest are usually in the C-terminus of the  $\alpha_1$ -domain and N-terminus of the  $\alpha_2$ -domain. The region 60-85 of the  $\alpha_1$ -domain, more particularly 62 to 85 or 72-82 are found to be of particular interest. Regions of the  $\alpha_2$ -domain of particular interest are amino acids 72-75 separated by 2 to 4 amino acids from amino acids 79-82.

It is found that the amino acids from 83 to 85 of the native MHC Class I antigens are of interest. For both MHC Class I D and K, or analogous HLA-B or C, the sequence is R Y Y. Peptides derived from the native MHC sequence of interest may comprise, desirably at their C-terminal end, the sequence R Y Y, desirably R Y X, where X may be any amino acid, particularly alanine, or the chemical group NH<sub>2</sub> or other amino derivative. Peptides derived from the native MHC sequence of interest may include at least about 20, usually at least about 15, and preferably not more than about 10 amino acids on either side of the sequences indicated above, desirably having at least 5 amino acids at the N-terminal side, and more desirably not having more than about 5-amino acids at the C-terminal side of the native sequence. Desirably, the total number of amino acids will not exceed 24, preferably will not exceed about 18 with the sequences indicated above. Increasing the extent of the sequence does not significantly affect the activity, but rather primarily affects the ability to synthesize such peptides and to provide additional characteristics such as solubility, stability, and the like.

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Also of interest is the region from about amino acid 30 to amino acid 45, more particularly 32 to 40, particularly an oligopeptide of at least four amino acids, more usually at least about six amino acids, and preferably at least 5 about eight amino acids, where the sequence includes a tetramer involving an acidic amino acid and a basic amino acid separated by one neutral amino acid, particularly a neutral amino acid of at least five carbon atoms and one of the acidic or basic amino acids is flanked by a neutral 10 amino acid. Of particular interest is where the intervening neutral amino acid is an aromatic or aliphatic hydrocarbon amino acid, e.g. glycine or phenylalanine.

A large number of surface membrane proteins are involved with the transduction of signals and serve as 15 receptors for a wide variety of ligands. For the most part, receptors are defined by the ligand which activates the receptor for transduction or serves to endocytose the ligand. These receptors include endocrine, paracrine and autocrine receptors, adrenergic receptors, lipoprotein 20 receptors (including the low-density lipoprotein (LDL) and scavenger receptors), opiate receptors, and steroid receptors. These receptors also include surface protein receptors for asialoglycoprotein, insulin, somatostatin, somatotropins, growth factors, such as growth hormone, 25 platelet derived growth factor, insulin-like growth factor, epidermal growth factor (EGF),  $\alpha$ -transforming growth factor, nerve growth factor, fibroblast growth factor, somatomedin, vasopressin, prostaglandins, eosinophil chemotactic factor, acetylcholine, thyroxine (TSH), epinephrine; endorphins, 30 enkephalins and dynorphins; neuropeptid Y, oxytocin, transferrin, substance P, lymphokines, such as IL-1, -2, -3 and -4, etc.; colony stimulating factors, such as GM-CSF, M-CSF, G-CSF, etc.; lipoproteins, such as LDL; and steroids, such as estrogen, androgen, glucocorticoids, 35 corticosteroids, etc. Additional receptors include those receptors employed in the receptor-mediated endocytosis of

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certain microbial pathogens (e.g. viruses). Of particular interest are receptors which are either internalized or are recycled, that is, internalized into the cytoplasm and optionally returned to the plasma membrane surface.

5 Illustrative of these receptors are the receptors for insulin, EGF, LDL, transferrin, interleukins, and asialoglycoprotein.

Modulation of the MHC Class I antigen activation may be achieved in a variety of ways. The number of MHC antigen 10 molecules at the surface can be increased or decreased by employing compounds which activate or inhibit Class I antigen production. These compounds include interferon, dimethyl sulfoxide (DMSO), tetradecylphorbyl acetate (TPA), and retinoic acids. Instead of altering the MHC Class I 15 antigen population at the surface, the effective concentration of Class I antigen for interacting with receptors may be reduced. It is noted that viral infections deplete Class I antigens at the surface and in appropriate situations may be used for this purpose.

20 Alternatively, one may modulate the interaction between MHC Class I antigen and surface receptors by employing antibodies to the MHC Class I antigen  $\alpha_1$ - or  $\alpha_2$ -domain, particularly the  $\alpha_1$ -domain, which may affect the ability of MHC to modulate surface expression of a receptor. Either 25 polyclonal or monoclonal antibodies may be employed, particularly monoclonal. Alternatively, one may employ the monoclonal antibodies specific for the  $\alpha_1$ -domain to be used as immunogens for the production of anti-idiotype antibodies, which will mimic the conformation of the Class 30 I antigen epitope to which the monoclonal antibody binds. Thus, the anti-idiotype antibody may act as a substitute Class I antigen and may serve to block immune responses which are directed against self-antigens of the host, as in autoimmune diseases. The whole antibodies need not be 35 employed, the variable region sufficing, or larger fragments such as Fab or  $F(ab')_2$ , Fab', etc.

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The antibodies may be prepared in accordance with conventional techniques. Particularly, the Class I antigen may be used as an immunogen and injected into an appropriate host, conveniently a mouse, for initiating an immune response. One or more booster injections may be employed at intervals of two or more weeks. Two to three days after the last injection, the animal host may be sacrificed, the spleen isolated, and the B-lymphocytes immortalized. Various techniques exist for immortalization, conveniently fusion with a myeloid cell, followed by selecting for hybridomas and screening under limiting dilution conditions for hybridomas producing antibodies having the desired characteristics. Thus, in the present situation the Class I antigen or, in the case of the anti-idiotype, the antibody with binding specificity to the domain of interest could be used in a competition assay for screening.

Instead of employing antibodies, oligopeptides may be employed which are capable of altering, either directly or indirectly, the interaction between MHC antigens and surface receptors. Altering of MHC-surface receptor interaction may be achieved by, for example, binding to the  $\alpha$ -helix of MHC Class I antigen. Such binding may occur through a substantially self-self interaction between the  $\alpha$ -helix of the native MHC antigen and a peptide having substantially the same amino acid sequence as the native MHC  $\alpha$ -helix. By modifying the peptide sequence, for example by substitutions, deletions or insertions, where usually from 1 to 3, usually from 1 to 2, amino acids are involved, the activity of the peptide may be varied (i.e. enhanced).  
"Non-conservative substitutions" is intended to include those substitutions which substantially differ as to polarity and/or size In Table 1 each line indicates substitutions considered to be conservative substitutions.

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Table 1

**Neutral**

**Aliphatic**

**Non-polar**

5	small	G, A (P)
	large	V, I, L

**Polar**

Oxy or Thio	S, T, C, M
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Amide	N, Q
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10 Aromatic	F, W, H, Y
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**Charged**

Acidic	D, E
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Basic	K, R
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( ) intends that the amino acid will normally not be used  
 15 as a substitute for other amino acids on the same line.

While not wishing to be bound to the theory, it appears that the peptides are involved with inhibiting internalization of receptors by blocking interaction of MHC Class I molecules with receptors. In this manner, the 20 lifetime of the ligand-receptor complex on the cell surface is extended, so that one observes an enhanced activity as a result of binding of the ligand to the receptor. In addition, there may be other effects of the peptide, such as allosteric effects, which may enhance 25 binding affinity of the ligand and provide activation effects (where the peptide results in activation of the receptor), so as to provide for transduction of a signal into the cytoplasm, or other effects, where the sum total of the result is an enhanced effect as compared to the 30 absence of the peptide.

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In a variety of disease states, the disease results from reduced presence of a particular receptor at the surface or reduced affinity of the receptor for the ligand. In this situation, one could reduce the density 5 of the Class I MHC antigen or provide peptide at an appropriate concentration, so as to facilitate an increase in the amount of receptor presented on the cell surface and thus cause an increase in the probability of receptor activation and the concomitant receptor response.

10 Conditions such as diabetes, Graves disease, arthritis, ankylosing spondylitis, Reiter's disease, analgesia, viral disease, etc., may be treated where the disease is associated with inadequate receptor response.

Alternatively in other situations, where one wishes 15 to diminish the receptor response, one might wish to down regulate receptor binding of ligand. Illustrative of such conditions are neoplasia, arthritis, lupus erythematosus, etc., where it is desirable to reduce the response to growth factors or other secreted factors which encourage 20 proliferation or other undesirable response. In this situation, one may treat the target cells with a drug which would enhance the population of Class I antigens at the surface.

The subject peptides may affect one activity of the 25 receptor differently from a different activity. For example in the case of the insulin receptor, peptides mediate an increase in glucose uptake in whole cells and while the same peptides also diminish the tyrosine kinase activity of purified receptor. Thus, the subject peptides 30 may selectively modify a receptor having a plurality of activities.

As already indicated, Class I antigen depletion may also be achieved using antibodies or oligopeptides which either bind to the Class I antigen, thereby inhibiting 35 interaction with the receptor, or bind to the receptor, thereby inhibiting interaction with the Class I antigen.

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These compounds can be prepared by employing sequences comparable to polymorphic sequences, particularly in the α-domain of the Class I antigen. Of particular interest are oligopeptides comprising at least a portion of one of  
5 the following sequences, where the oligopeptides comprise as the active sequence, at least six amino acids, usually at least eight amino acids, more usually at least about 11 amino acids, and fewer than 40 amino acids, more usually fewer than 30 amino acids, preferably, not more than about  
10 25 amino acids, preferably being from about 8 to 25 amino acids, more preferably about 8 to 24 amino acids. It is understood that up to five, more usually up to about three substitutions or deletions may be made in the subject sequences, where the change will not be more than about 20  
15 number %, usually not more than about 10 number % of the number of amino acids in the active sequence. Also the following sequences may be joined together either contiguously or by bridges of not more than about 20 amino acids, more usually not more than about 10 amino acids.  
20 Furthermore, where the sequences overlap, it is intended that the overlapping sequences not be repeated, but rather that the non-overlapping sequences be joined in proper sequence.

The oligopeptide will have at least six amino acids  
25 which are the same, or substantially the same as, a sequence included in the following sequence.

1. W aa<sup>52</sup> E Q aa<sup>55</sup> aa<sup>56</sup> G P E Y W
2. W aa<sup>61</sup> aa<sup>62</sup> aa<sup>63</sup> T aa<sup>65</sup> aa<sup>66</sup> aa<sup>67</sup> K aa<sup>69</sup> aa<sup>70</sup> aa<sup>71</sup> Q
3. W aa<sup>61</sup> aa<sup>62</sup> aa<sup>63</sup> aa<sup>64</sup> aa<sup>65</sup> aa<sup>66</sup> aa<sup>67</sup> K aa<sup>69</sup> aa<sup>70</sup> aa<sup>71</sup> aa<sup>72</sup>  
30 aa<sup>73</sup> aa<sup>74</sup> aa<sup>75</sup> aa<sup>76</sup> aa<sup>77</sup> aa<sup>78</sup> aa<sup>79</sup> aa<sup>80</sup> aa<sup>81</sup> aa<sup>82</sup> aa<sup>83</sup> aa<sup>84</sup>  
aa<sup>85</sup>
4. G N E Q aa<sup>73</sup> aa<sup>74</sup> R V aa<sup>77</sup> aa<sup>78</sup> R aa<sup>80</sup> aa<sup>81</sup> aa<sup>82</sup> R Y aa<sup>85</sup>  
wherein:  
aa<sup>52</sup> is a neutral aliphatic amino acid of from 4 to 6  
35 carbon atoms, particularly V, I, L or M, more particularly

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V or I;

aa<sup>55</sup> is any charged amino acid, particularly K, R, D, or E, more particularly K or E;

5 aa<sup>56</sup> is a charged amino acid, particularly D, E, K or R, more particularly E or K;

aa<sup>57</sup> is D or E;

aa<sup>58</sup> is K, R, G, or A, particularly R or G;

10 aa<sup>59</sup> is any aliphatic amino acid other than basic of from 4 to 6 carbon atoms, particularly D, E, I, L, V, N, or Q, more particularly E, N, or Q;

aa<sup>60</sup> is S, T, or M, particularly T;

aa<sup>61</sup> is any polar or basic amino acid of 4 to 6 carbon atoms, particularly N, Q, K or R, more particularly Q;

15 aa<sup>62</sup> is any aliphatic amino acid of from 4 to 6 carbon atoms, particularly L, I, V, K, R, N, or Q, more particularly K, I or N;

aa<sup>63</sup> is any neutral aliphatic or aromatic amino acid, particularly G, A, L, V, I, S, T, M, C, F, Y, N, or Q,

20 more particularly C, S, Y, or M;

aa<sup>64</sup> is K or R, particularly K;

aa<sup>65</sup> is any aliphatic neutral amino acid other than A or any acidic amino acid, particularly D, E, G, S, T, or M, particularly G or T;

25 aa<sup>66</sup> is any aliphatic amino acid, neutral, polar, or basic (other than acidic) from 3 to 6, usually 4 to 6 carbon atoms, particularly N, Q, K, R, S, or T, more particularly N, Q, or K;

aa<sup>67</sup> is any aliphatic amino acid other than basic, 30 usually from 2 to 5 carbon atoms, particularly G, A, S, T, D, or E, more particularly A, T or E;

aa<sup>68</sup> is N or Q, particularly Q;

aa<sup>69</sup> is S, T, F, Y, H, or W, particularly T or S;

aa<sup>70</sup> is F, Y, H, or W, particularly F;

35 aa<sup>71</sup> is K or R, particularly R;

aa<sup>72</sup> is an aliphatic amino acid other than basic of

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from 4 to 6 carbon atoms, particularly D, E, V, I, or L, more particularly E or V;

aa<sup>77</sup> is a polar aliphatic amino acid of from 3 to 6 carbon atoms particularly N, Q, S, T, D, or E, more

5 particularly N, D or S;

aa<sup>78</sup> is a non-polar aliphatic amino acid of from 3 to 6 carbon atoms, particularly A, P, V, I, or L, more particularly L;

aa<sup>79</sup> is K or R, particularly R;

10 aa<sup>80</sup> is a neutral aliphatic amino acid, other than A, of from 3 to 6 usually 4 to 6 carbon atoms, particularly, S, T, N, Q, I, V or L, more particularly N, T, or I;

aa<sup>81</sup> is an aliphatic non-polar amino acid, other than A, particularly G, L, I, or V, more particularly L;

15 aa<sup>82</sup> is a non-acidic aliphatic amino acid other than A, of from 2 to 6, usually 5 to 6, carbon atoms, particularly K, R, G, L, I, or V, more particularly L;

aa<sup>83</sup> is a non-acidic aliphatic amino acid other than A, of from 2 to 6 carbon atoms, particularly K, R, G, L, 20 I, or V, more particularly G or R;

aa<sup>84</sup> is an aromatic amino acid, particularly F, Y, H, or W, more particularly Y; and

aa<sup>85</sup> is either an aromatic amino acid, particularly F, Y, H, or W, more particularly Y, or a non-polar 25 aliphatic amino acid, preferably A.

Preferably, there will usually not be more than three mutations in the above sequence as substitutions, deletions, or insertions.

Of particular interest is an amino acid sequence of 30 at least 6, usually at least 17, amino acids contained within the following sequence.

W D/E R aa<sup>63</sup> T Q/R aa<sup>66</sup> aa<sup>67</sup> K aa<sup>69</sup> aa<sup>70</sup> aa<sup>71</sup> Q aa<sup>73</sup> F R  
V/E aa<sup>77</sup> L R aa<sup>80</sup> L/A L/R G/R Y aa<sup>85</sup>

wherein:

35 aa<sup>63</sup> is E, I, or N;

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aa<sup>66</sup> is I, N, or K, particularly I;  
aa<sup>67</sup> is A, C, S, M, or Y, particularly Y or C;  
aa<sup>69</sup> is G, A, T, or P, particularly A or T;  
aa<sup>70</sup> is Q, N, or K;  
5 aa<sup>71</sup> is A, E, or T;  
aa<sup>73</sup> is T, W or S  
aa<sup>77</sup> is N, S, or D;  
aa<sup>80</sup> is I, N, or T, particularly T; and  
aa<sup>85</sup> is any amino acid, preferably Y or A, more  
10 preferably A;  
and where when two amino acids are indicated at a particular site, either amino acid may be employed interchangeably. Up to three of the amino acids may be subject to conservative or non-conservative changes, there  
15 being from 0 to 2 deletions or insertions of from 1 to 2 amino acids.

Compositions of interest include an amino acid sequence contained within the following sequence:

N/Q S/T aa<sup>74</sup> K/R aa<sup>76</sup> aa<sup>77</sup> aa<sup>78</sup> K/R S/T I/L/T I/L/T  
20 wherein:

aa<sup>74</sup> is F, Y, or W, particularly F;  
aa<sup>76</sup> is any is a non-acidic aliphatic amino acid other than A, particularly V;  
aa<sup>77</sup> is N, S, or D;  
25 aa<sup>78</sup> is any is a non-acidic aliphatic amino acid other than A, particularly L;  
and where more than one amino acid is indicated at a sire, any one amino acid may be employed interchangeably with the other two.  
30 The subject oligopeptides are further characterized by helix formation resulting in a structure capable of aggregation; positive and negative maxima in a circular dichroic spectrum with a profile similar to the peptide consisting of amino acids 69 to 85 of the  $\alpha$ -helix of the  
35 D<sup>k</sup> MHC Class I antigen; and the ability to bind to Class I

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MHC antigen.

The oligopeptides may be provided in a variety of ways, being joined to non-wild-type flanking regions, as fused proteins, joined by linking groups or directly covalently linked through cysteine (disulfide) or peptide linkages. The oligopeptides may be joined to a single amino acid at the N- or C-terminus or a chain of amino acids. The fused peptides may be extended to provide convenient linking sites, e.g. cysteine or lysine, to enhance stability, to bind to particular receptors, to provide for site-directed action, to provide for ease of purification, to alter the physical characteristics (e.g. solubility, charge, etc.), to stabilize the conformation, etc. The oligopeptide may be N-terminal, C-terminal or internal. The oligopeptide may be linked through a variety of bifunctional agents, such as maleimidobenzoic acid, methyldithioacetic acid, mercaptobenzoic acid, S-pyridyl dithiopropionate, etc. The oligopeptides may be linked to proteins to provide immunogens for the production of antibodies or to provide for site-directed action. The oligopeptides may be linked, particularly by an intracellular cleavable linkage, to antibodies for site directed action. For conjugation techniques, see, for example, U.S. Patent Nos. 3,817,837; 3,853,914; 3,850,752; 3,905,654; 4,156,081; 4,069,105; and 4,043,989, which are incorporated herein by reference.

The oligopeptides may also be modified by incorporation into the lumen of vesicles, e.g. liposomes, which in turn may be bound to ligands or receptors for direction to particular cells or tissue.

The oligopeptides may be employed in a variety of ways. For therapy, they may be administered topically or parenterally, e.g. by injection at a particular site, for example, subcutaneously, intraperitoneally, intravascularly, or the like or transdermally, as by electrotransport. The oligopeptides may also be administered subcutaneously as a self-aggregating gel so as to concentrate peptide at the

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desired site of action or to provide a depot of active peptide for slow release over an extended period. Furthermore, gels of peptides may serve as reservoirs for delivery of other, additional drugs such as insulin, EGF, 5 growth hormones, and the like. Delivery of drugs in peptide gels would concentrate and retain the drug at a local site of action and provide for slow release of the drug over an extended period of time. Such delivery may decrease the dosage of drug required and may also decrease the number of 10 treatments necessary to achieve a therapeutic effect. Where the gel contains active peptides which provide for increased surface expression of a receptor which binds a drug contained in the gel, gels may serve as a method to provide for a synergistic therapeutic effect of peptide and drug.

15 The formulations will usually involve a physiologically acceptable medium, such as deionized water, saline, aqueous ethanol, phosphate buffered saline, and the like. The manner of formulation will vary depending upon the purpose of the formulation, the particular mode employed for 20 modulating the receptor activity, the intended treatment, and the like. The formulation may involve patches, capsules, liposomes, time delayed coatings, pills, or may be formulated in pumps for continuous administration. Because of the wide variety of modes of treatment, the varying 25 responses, the different disease states, and the like, no useful limits may be given for the concentration of the active components. These can be determined empirically in accordance with known ways. See, for example Harrison's, Principles of Internal Medicine, 11th ed. Braunwald et al. 30 ed, McGraw Hill Book Co., New York, 1987.

The oligopeptides derived from the a-helix of Class I MHC antigens may also find use in a drug screening assay. Such an assay may take advantage of the correlation between the ability of peptides to form micelles and gel-like 35 aggregates and to inhibit surface receptor internalization mediated by native MHC antigens. This correlation of

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aggregative characteristics with biological activity may support the theory that peptides derived from an  $\alpha$ -helix of Class I MHC actually bind to the portion of the Class I  $\alpha$ -helix from which it was derived. This interaction is 5 analogous to the self interaction of peptides having biological activity. Drug candidates which may be capable of inhibiting surface receptor internalization may then be identified by first screening the drug candidates for the ability to successfully disrupt peptide-peptide self 10 interactions. Alternatively, the screening assay may involve competition of the drug candidate with peptide for association with either Class I MHC antigen, receptor, a saturable cell-surface binding site, or an accessory molecule(s) involved in surface receptor internalization. 15 Drug candidates which affect receptor internalization may also be identified by screening drugs for the ability to either enhance or reduce the effect of peptides on the internalization of a selected surface receptor.

In one embodiment of the screening assay, a peptide 20 derived from Class I MHC antigen and having modulatory activity or a substantially purified Class I MHC antigen is non-diffusably bound to an insoluble support having isolated sample receiving areas (e.g. a microtiter plate). The insoluble supports may be made of any composition to 25 which peptide, Class I MHC antigen, or other protein can be bound, is readily separated from soluble material, and is otherwise compatible with the overall method of screening. The surface of such supports may be solid or porous and of any convenient shape. Examples of suitable 30 insoluble supports include microtiter plates, membranes and beads. These are typically made of glass, plastic (e.g. polystyrene), polysaccharides, nylon or nitrocellulose. Microtiter plates are especially 35 convenient because a large number of assays can be carried out simultaneously, using small amounts of reagents and samples. The particular manner of binding of the peptide,

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Class I MHC antigen or other protein is not crucial so long as it is compatible with the reagents and overall methods of the invention, maintains the activity of the peptide and is nondiffusible. Following binding peptide 5 or Class I MHC antigen, excess unbound material is removed by washing. The sample receiving areas may then be blocked through incubation with bovine serum albumin (BSA), casein or other innocuous protein.

The drug candidate and varying concentrations of the 10 oligopeptide are added to each of the sample receiving areas containing support-bound peptide or Class I MHC antigen. The oligopeptide added is of substantially the same amino acid sequence as the oligopeptide bound to the support and is labeled. The oligopeptides could be 15 labeled, directly or indirectly, with a label which provides a detectable signal, e.g. radioisotope, fluorescers, enzyme, particle, chemiluminescer, etc. Positive controls for binding of active peptide and competitive binding of active peptide may include samples 20 containing labeled active peptide alone and a mixture of labeled active peptide and unlabeled active peptide, respectively. Samples containing labeled active peptide and unlabeled inactive peptide which does not aggregate with the bound peptide may serve as a negative control for 25 competitive binding with peptide. Preferably all control and test samples are performed in at least triplicate to obtain statistically significant results. Incubation of all samples is for a time sufficient for the binding of the labeled active peptide to the support-bound peptide. 30 Following incubation, all samples are washed free of non-specifically bound material and the amount of bound, labeled peptide determined. For example, where a radiolabel is employed in labeling the peptide, the samples may be counted in a scintillation counter to 35 determine the amount of bound, labeled peptide.

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In test samples containing the drug candidate, if the amount of labeled active peptide bound to the support-bound peptide or Class I MHC antigen is in the range of values of the positive control samples for competitive binding and is significantly less than binding of labeled peptide in the active peptide alone and negative control samples for competitive binding, then the drug candidate in the test sample is able to successfully competitively bind the support-bound peptide or Class I MHC antigen.

5 Drug candidates capable of such competitive binding may mediate modulation of cell surface expression of a receptor with a peptide-like activity.

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In an alternative embodiment, a labeled antibody having binding specificity for Class I MHC antigen and/or a peptide derived from is substituted for the labeled active peptide in the screening assay described above. This antibody may be a monoclonal or polyclonal antibody which competitively binds native Class I MHC antigen in the presence of peptide having modulatory activity. This antibody may also block aggregative interactions between peptides having modulatory activity by competitively binding to the peptides. The antibody may have binding specificity for the same or a different epitope than that with which biologically active peptides may bind during interaction with Class I MHC antigen or peptide aggregation. The antibody may be labeled, directly or indirectly, with a label which provides a detectable signal, e.g. radioisotope, fluorescers, enzyme, particle, chemiluminescer, etc. Following binding of the active peptide or Class I MHC antigen to the support, removal of excess peptide by washing and blocking of the sample receiving areas, test samples containing varying relative concentrations of a drug candidate and the labeled antibody are added. Positive controls for binding of the labeled antibody and competitive binding of labeled antibody and active peptide may include samples containing labeled antibody alone and a mixture of labeled antibody and

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unlabeled active peptide, respectively. Samples containing labeled antibody and unlabeled inactive peptide which does not aggregate with either the bound peptide or Class I MHC antigen may serve as a negative control for competitive binding with peptide. Preferably all control and test samples are performed in at least triplicate to obtain statistically significant results. As described above, incubation of all samples is for a time sufficient for the binding of the labeled active peptide to the support-bound peptide. Following incubation, all samples are washed free of non-specifically bound material and the amount of bound, labeled antibody determined. Drug candidates which successfully compete with the labeled antibody for association with the peptide bound or Class I MHC antigen to the support may mediate modulation of cell surface expression of a receptor with peptide-like activity.

In test samples containing the drug candidate, if the amount of labeled antibody bound to the support-bound peptide is in the range of values of the positive control samples for competitive binding and is significantly less than binding of labeled antibody in the antibody alone and negative control samples for competitive binding, then the drug candidate in the test sample is able to successfully competitively bind the support-bound peptide or Class I MHC antigen. Drug candidates capable of such competitive binding may mediate modulation of cell surface expression of a receptor with a peptide-like activity.

In a further embodiment, the screening assay may employ whole cells with saturable binding sites for either a biologically active peptide or for an antibody with binding specificity for Class I MHC antigen. Following the seeding of cells to an insoluble support, test samples containing varying relative concentrations of a drug candidate and either a labeled peptide having modulatory activity or a labeled antibody having binding specificity to MHC Class I antigen are added. Positive controls for binding of the

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labeled peptide or labeled antibody may include samples containing labeled peptide or labeled antibody alone. Positive controls for competitive binding may include samples containing unlabeled active peptide mixed with either labeled peptide or labeled antibody. Samples containing unlabeled inactive peptide (which does not aggregate with either the bound peptide or Class I MHC antigen) mixed with either labeled peptide or labeled antibody may serve as a negative control for competitive binding with whole cells. Preferably all control and test samples are performed in at least triplicate to obtain statistically significant results. Incubation of all samples is for a time sufficient for the binding of the labeled active peptide or labeled antibody to whole cells. Following incubation, all samples are washed free of non-specifically bound material and the amount of bound, labeled antibody or labeled peptide determined. Drug candidates which successfully compete with either the labeled peptide or labeled antibody for association with the saturable binding site of cells may mediate modulation of cell surface expression of a receptor with peptide-like activity.

In test samples containing the drug candidate, if the amount of labeled peptide or labeled antibody bound to the cells is in the range of values of the positive control samples for competitive binding and is significantly less than binding of labeled peptide or labeled antibody in the antibody alone and negative control samples for competitive binding, then the drug candidate in the test sample is able to successfully competitively bind the saturable binding site present on the cell surface. Drug candidates capable of such competitive binding may mediate modulation of cell surface expression of a receptor with a peptide-like activity.

Alternatively, the screening assay may examine the ability of a drug candidate to inhibit internalization of a selected receptor known to be affected by interaction with

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Class I MHC. For example, whole cells may be seeded on a soluble support. Test samples containing the drug candidate would then be added in varying concentrations to the cell monolayers. Positive controls may include samples having either biologically active peptide or an antibody with binding specificity for Class I MHC antigen. Negative controls (basal level) may include untreated samples, biologically inactive peptide or antibody which does not bind MHC Class I antigen. Following incubation for a time known to be sufficient for detection of an effect in positive control samples, labeled ligand which binds to the receptor of interest is added to all samples. For example, if the receptor of interest is the insulin receptor, then the labeled ligand may be  $^{125}\text{I}$ -insulin. The samples are incubated for a time sufficient for binding of the labeled ligand to receptors and then washed free of non-specifically bound ligand. In test samples containing the drug candidate, if the amount of labeled ligand bound to the cells is in the range of values of the positive control samples and is significantly more than binding of labeled ligand in the negative control samples, then the drug candidate in the test sample may mediate modulation of cell surface expression of a receptor with a peptide-like activity.

In a further alternative embodiment, the screening assay may examine the ability of a drug candidate to inhibit gel formation by active peptides. A solution containing an active peptide may be mixed with varying concentrations of the drug candidate of interest. A solution of active peptide alone may serve as a positive control for gel formation. A solution of differing ratios of active peptide and inactive, non-aggregative peptide may serve as a negative control for gel formation. The molar concentration of inactive peptide required to disrupt gel formation represents that level at which disruption of active peptide gel formation is non-specific (i.e. due to dilution of

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active peptide solution). Drug candidates which inhibit gel formation of active peptide at a molar concentration significantly lower than that associated with non-specific disruption of gel formation may then be capable of

5 modulating surface receptor response with peptide-like activity. This embodiment of the screening assay may provide a quick, qualitative method for identifying those drug candidates which competitively bind peptide and thus interrupt peptide-peptide interactions.

10 The oligopeptides of this invention may be prepared in accordance with conventional techniques, such as synthesis (for example, use of a Beckman Model 990 peptide synthesizer or other commercial synthesizer), recombinant techniques, or the like. See, for example, Maniatis et al., Molecular

15 Cloning: A Laboratory Manual, CSH Laboratory, Cold Spring Harbor, New York, 1982.

The following examples are offered by way of illustration and not by way of limitation.

EXPERIMENTAL

20 Example 1. MHC Class I Regulation of Surface Expression of Insulin Receptor

Mutant line, R1E, derived from the murine thymoma line R1 by chemical mutagenesis (Parns and Seidman, Cell (1982) 29:661-669; Allen et al., Proc. Natl. Acad. Sci. USA (1986) 25:7447-7451) expresses, in contrast to R1, none of the parental H<sub>2</sub><sup>k</sup> haplotype antigens of the C58 strain of origin due to the lesions induced in the b<sub>2</sub>-microglobulin (b<sub>2</sub>m) gene in R1E. Specific insulin binding to R1E murine thymoma cells and various R1E transfectants was determined, with the 30 binding being performed as described in Gavin et al., J. Biol. Chem. (1973) 248:2202-2207 and Due et al., Diabetologia (1985) 28:749-755.

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All cells were cultivated in RPMI-1640 with 15% fetal calf serum (FCS) and with the various additives as indicated (Allen et al., supra (1986)). Prior to insulin binding assays, the cells were seeded in RPMI-1640 with 10% FCS and  
5 at a density of  $2 \times 10^4$  cells/ml, harvested three days later, viability was assured to be >95% by trypan blue dye exclusion, and the cells subsequently resuspended in assay buffer for insulin binding at a concentration of  $7.5 \times 10^7$  cells/ml.  $^{125}\text{I}$ -labeled human insulin in a final  
10 concentration of 50 pM (labeled in the A14 position and obtained from NOVO A/S, Denmark) was added and the cells incubated for 90 min at 18°C in a shaking water bath. Two mls ice-cold assay buffer were added at the end of incubation, the cells centrifuged at 300 g for 5 min,  
15 centrifuged at 100 g for 10 min and the amount of  $^{125}\text{I}$ -insulin in the pellet counted in a g-counter. Non-specific binding was estimated as the amount of  $^{125}\text{I}$ -insulin binding in the presence of  $10^{-6}\text{M}$  unlabeled insulin, and specific insulin binding calculated as the difference in binding of  
20  $^{125}\text{I}$ -insulin with and without unlabeled insulin. Specific binding <1% was estimated to be non-specific considering the Scatchard plots and the specific binding as related to cell number.

Scatchard plots were complied, with each point  
25 representing duplicate or triplicate samples, for each of lines R1, R1E, R1E/b<sub>2</sub>m, R1E/D<sup>b</sup>, R1E/b<sub>2</sub>m/K<sup>b</sup>, R1E/b<sub>2</sub>m/D<sup>b</sup>, R1E/b<sub>2</sub>m/D<sup>b</sup>d, and R1E/b<sub>2</sub>m/D<sup>b</sup> - (1 + 2) with  $3 \times 10^7$  cells per sample and. The Scatchard plots were repeated 3-10 times for each determination. Only R1 and R1E/b<sub>2</sub>m/D<sup>b</sup> displayed  
30 applicable amounts of insulin receptor (IR). The curve observed shows that in addition to high affinity IR, these cells also have appreciable amounts of receptors with lower affinity for insulin, which may to some extent be due to the indirect effects of transfection and/or co-expression of  
35 other insulin binding receptors, such as those for insulin-like growth factor I (IGF-I) (Rechler and Hessley In

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Polypeptide Hormone Receptors (ed B.I. Posner) pp. 227-297,  
Marcel Dekker, New York (1985)).

R1 murine thymoma cells have a cell surface density of IR comparable to other lymphocyte cell populations in contrast to the human IM-9 cell line, an Epstein-Barr virus transformed cell line with exceptionally high amounts of non-functional IR often used for insulin assays. It was accordingly necessary in the R1/R1E system to use comparatively high amounts of cells per sample. Titration of specific insulin binding as related to cell number demonstrated that the optimal cell number per sample for specific insulin binding was  $7 \times 10^7$  cells, an impractical number of cells to use on all Scatchard plots. The curves for R1 and R1E/b<sub>2</sub>m/K<sup>b</sup> show that these two lines did not express significant amounts of IR.

Insulin receptor mRNA in R1, R1E and R1E transfectants was determined as follows. Total RNA was isolated from cells as per Chirgwin et al., Biochemistry (1979) 18:5294-5299, and poly A+ RNA selected as per Maniatis et al., 20 Molecular Cloning: A Laboratory Manual CSH Laboratory, Cold Spring Harbor, New York, (1982). For Northern blot analysis 5 µg of poly A+ selected murine liver mRNA was fractionated on a 1.0% agarose-formaldehyde gel (Church and Gilbert, Proc. Natl. Acad. Sci. USA (1984) 81:1991-1995) and blotted 25 on a Zeta-probe nylon membrane (BioRad Laboratories, Richmond, CA). Insulin receptor-specific sequences were detected by hybridization with a synthetic DNA oligonucleotide representing amino acids 732-741 inferred from the insulin receptor cDNA precursor (Ullrich, et al., 30 Nature (1985) 313:756-761). Hybridization and washing conditions were performed as per Church and Gilbert, supra (1984), except that hybridization and washes were at 45°C. Approximately 1 µg and 1:10 dilution of poly A+ selected mRNA from mouse liver and appropriate cell lines was spotted 35 on the Zeta-Probe membranes and hybridized as above. Molecular weight markers for Northern blot analysis were

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purchased from Bethesda Research Laboratories (Bethesda, MD). A predominant species of 4.8 kb from mouse liver hybridized to the human insulin receptor oligonucleotide. This species was also noted by Ullrich et al., supra (1985),  
5 in human placental mRNA with radiolabeled cloned human insulin receptor cDNA sequences.

The surface proteins of the various cell lines were screened using fluorescent labeled monoclonal antibodies and a fluorescence-activated cell sorter (FACS). The results  
10 are summarized in Table 2.

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Table 2  
**Fluorescence-Activated Cell Sorter (FACS) Analysis**  
R1, R1E and the Transfectants for Expression of H-2 and b

		No. FITC-molecules/cell $\times 10^{-3}$ <sup>a,b</sup>						
		Cell	H-2 specificity	K <sup>k</sup> D <sup>k</sup>	K <sup>k</sup>	K <sup>b</sup>	D <sup>k</sup>	D <sup>b</sup>
		b <sub>2</sub> m <sup>b</sup>	b <sub>2</sub> m					
		Lines <sup>c</sup>	monoclonal	(28-8-6)	(11.4)	(20-8-4)		
5		(15-5-5)	(28-14-8)					
			antibodies <sup>a</sup>					
		R1		750	505	<10	435	16
10	<10			175				
	R1E			25	<10	<10	<10	
	<10			<10	<10			
	R1E/b <sub>2</sub> m			290	80	15	90	15
	225			80				
15	R1E/D <sup>b</sup>			145	30	<10	30	
	570			<10	<10			
	R1E/b <sub>2</sub> m/D <sup>b</sup>			370	115	10	170	
	1240			450	390			
	R1E/b <sub>2</sub> m/K <sup>b</sup>			400	215	410	260	
20	<10			510	425			
	R1E/b <sub>2</sub> m/D <sup>d</sup>			345	170	10	320	
	250			200	105			
	R1E/D <sup>b</sup> -(1+2)			190	70	<10	50	
	825			<10	<10			
25	R1E/b <sub>2</sub> m/D <sup>b</sup> -(1+2)			350	105	10	130	
	880			210	160			

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Legend to Table 2

<sup>a</sup> The monoclonal antibodies used for screening have been described (Ozato et al., Transplantation (1982) 34:113-120). For staining,  $10^6$  cells were incubated with 1  $\mu$ g antibody/ml  
5 at 4°C with fluorescein-isothiocyanate (FITC) conjugated rabbit anti-mouse polyclonal antibody (purchased from DAKO, Denmark). The cells were washed twice in PBS and analyzed. Cells incubated with FITC-conjugated secondary antibody served as negative controls. A shift of >15 channel numbers  
10 on the linear fluorescence scale was considered significant; all samples were analyzed both on a logarithmic and linear fluorescence scale. The absolute number of bound FITC molecules per cell was estimated as described (Due et al., *supra*, (1985)). It should be noted that comparison of FACS  
15 data to estimate the relative proportion of different H-2 molecules only is reasonable when the same primary antibody is used.

<sup>b</sup> Expression of H-2 was determined for all lines examined both by solid phase radioimmunoassay (RIA) with  $^{125}$ I-labeled  
20 protein A as secondary reagent and by FACS as described above. Briefly, RIA assays (Weiss et al., Nature (1984) 310:650-655) were done by plating  $5 \times 10^5$  cells (96 well microtiter plate) in 50  $\mu$ l diluted antibody in MEM + 10% FCS was then added. The cells were incubated 4 hr at 4°C,  
25 pelleted and washed with MEM + 10% FCS.  $^{125}$ I-Protein A was added (Amersham) to 100,000 cpm and incubated for 16 hr at 4°C. Cells were pelleted and washed three times with MEM + 10% FCS before counting in a Beckman Gamma Counter. All samples were done in duplicate and with less than 5%  
30 variation on all samples counted.

<sup>c</sup> The R1, R1E lines, the H-2K and H-2D genes, and the procedures for transfection have been described in detail previously (Allen et al., Proc. Natl. Acad. Sci. USA (1986) 83:7447-7451; Goodenow et al., Science (1982) 215:677-679).

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The various designations indicate: R1E/b<sub>2</sub>m, R1E transfected with b<sub>2</sub>m, R1E/D<sup>b</sup>; transfected with D<sup>b</sup>; R1E/b<sub>2</sub>m/D<sup>b</sup>, transfected with b<sub>2</sub>m and D<sup>b</sup>; R1E/b<sub>2</sub>m/K<sup>b</sup> transfected with b<sub>2</sub>m and K<sup>b</sup>; R1E/b<sub>2</sub>m/D<sup>b</sup> + a-D<sup>b</sup>, R1E transfected with b<sub>2</sub>m and D<sup>b</sup>, but with  
5 the D<sup>b</sup> cell surface antigen down-regulated due to the presence of a monoclonal antibody to D<sup>b</sup>; R1E/b<sub>2</sub>m/D<sup>b</sup>-(1+2), transfected with b<sub>2</sub>m and truncated D<sup>b</sup> gene, expressing only the a3-domain on the cell surface; R1E/b<sub>2</sub>m/D<sup>b</sup>-(1+2), transfected with truncated D<sup>b</sup>, expressing only the a3-  
10 domain.

Example 2. Study of MHC Class I Peptide Regulation of Purified Insulin Receptor

Peptides. The two MHC Class I derived peptides D<sup>k</sup>-(61-85), and K<sup>k</sup>-(61-85) are both from the same region of the a<sub>1</sub> 15 domain of the MHC Class I molecules (Klein, Natural History of the Major Histocompatibility Complex (Wiley, New York)). Both peptides were synthesized by Applied Biosystems, Inc., (Foster City, CA), and quality controlled by mass spectrometry.

20 The D<sup>k</sup>-(61-85), and K<sup>k</sup>-(61-85) peptides were iodinated for some experiments using carrier-free Na<sup>125</sup>I (Amersham) and iodobeads (Pierce) by incubating for 20 min, then purified by reversed-phase HPLC on a C<sub>18</sub> column (Beckman) in a linear 30-50% gradient of CH<sub>3</sub>CN in 5 mM trifluoroacetic acid (TFA).  
25 The <sup>125</sup>I-labeled peptide eluting first was stored at 4°C in 50% CH<sub>3</sub>CN/5 mM TFA. The labeled peptides were stable under these conditions for at least 3 months.

Control Peptides: ACTH-(1-24) (human), ACCK-33 (porcine), dynorphin A (porcine), b-endorphin-(1-27) 30 (camel), glucagon (human), and prosomatostatin-(1-32) (porcine) were all purchased from Peninsula Laboratories, Belmont, CA. The A-chain and B-chain of insulin (porcine) and glucagon-(1-21) (human) were obtained from Novo

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Industry, Denmark. ACTH-(1-24) was used as a routine control peptide.

Purified Insulin Receptor (IR). The purified human IR and the cloned cytoplasmic kinase domain (IRKD) have been 5 described (Ellis et al., (1988) Virology **62**:1634-39; Roth et al., (1986) J. Biol. Chem. **261**:3753-57). Briefly, the human IR was purified from placenta by immunoaffinity columns, using monoclonal antibodies and binding of IR to wheat germ agglutinin. The product was a tetramer with two heavy 10 chains, each  $\approx$ 130 kDa, and two light chains, each  $\approx$ 90 kDa.

Tyrosine Kinase Activity. The cytoplasmic, cloned IRKD was constructed from the IR sequence (Ebina et al., (1985) Cell **40**:747-758; Ullrich et al., (1985) Nature **313**:756-761) and expressed in insect cells by using a baculovirus 15 expression vector. The domain is soluble ( $M_r \approx$ 48 kDa) and the kinase activity is constitutively expressed in vitro. The IRKD was purified to homogeneity by immunoaffinity chromatography.

The procedures to measure kinase activity of the 20 purified IR and IRKD, as well as the effects of insulin, have been described elsewhere (Roth et al., (1986) *supra*). Briefly 5.0  $\mu$ l purified IR was mixed with 5.0  $\mu$ l insulin (final concentration 1.0  $\mu$ M) and the buffer (50 mM HEPES, pH 7.6, 150 mM NaCl, 0.1% Triton X-100) added to a final volume 25 of 20  $\mu$ l. When peptide was used, it was added in 5.0  $\mu$ l, the volume adjusted to 20  $\mu$ l by adding buffer, and the mixture incubated (1 hr, 4°C). After incubation, 10  $\mu$ l of a solution containing 2.5  $\mu$ Ci  $^{32}$ P-labeled ATP (3,000 Ci/mmol; g-labeled; Amersham) 50 mM HEPES, pH 7.6, 150 mM NaCl, 0.1% 30 Triton X-100, 37.5  $\mu$ M unlabeled ATP, 15 mM MgCl<sub>2</sub>, and 6 mM MnCl<sub>2</sub> was added to a final volume of 30  $\mu$ l. The mixture was then incubated for 30 min at 24°C.

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After incubation, 15  $\mu$ l sample buffer was added, the sample boiled for 5 min and then run on 10% SDS-PAGE overnight. The gel was dried, and autoradiograms processed with an exposure time of 5 to 10 hr. For quantitative estimates the b-subunit band of the IR and the IRKD bands were excised and counted dry (Cerenkov) in a scintillation counter.

Substrate phosphorylation was done with poly-([Glu,Tyr];4:1) (Sigma) as substrate. The substrate was added to a final concentration of 1.0 mg/ml and the phosphorylation assay was conducted as described above. Quantitative estimates were determined by excising the entire lane from just below the insulin receptor band to the 20-kD marker and quantitating the amount of associated radioactivity by either placing the sample in a scintillation counter or precipitating the sample with TCA and determining the amount of TCA precipitate-associated radioactivity. For the latter, a 5  $\mu$ l sample was dotted on to 3 MM paper (Whatman), washed 30 min in ice cold 10% TCA, boiled 10 min in 5% TCA, washed twice in distilled water, washed twice in ethanol, dried and counted.

Insulin Binding. Porcine monoiodinated [ $^{125}$ I]-insulin (iodinated at Tyr A14; 1,900-2,000 Ci/mmol) was obtained from NOVO Industry and Amersham. Unlabeled porcine insulin (NOVO) was dissolved in 10 mM HCl at 1 mM and stored immediately at -20°C.

The plate assay for insulin binding to its purified receptor has been described (Morgan and Roth (1985) Endocrinology 116, 1224-1226). Briefly, 50  $\mu$ l of affinity-purified rabbit anti-mouse IgG (Jackson Immuno Research Lab., Inc., West Grove, PA) (40  $\mu$ g/ml) in 20 mM NaHCO<sub>3</sub>, pH 9.6, was added to 96-well polyvinyl chloride (PVC) plates. The plates were incubated (17-20 hr, 4°C), washed thrice in 50 mM HEPES, pH 7.8, with 150 mM NaCl, 0.1% Triton X-100, 0.05% BSA, and 2  $\times$  10<sup>-8</sup> M monoclonal antibody (Amac, Inc.,

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Westbrook, ME) was added. After incubation (1 hr, 24°C), the plates were washed and insulin binding measured.

For binding measurements,  $^{125}\text{I}$ -insulin ( $3 \times 10^{-10}\text{M}$ ) was added together with increasing amounts of unlabeled insulin, 5 and incubated (90 min, 24°C), washed, and the amount of free and bound  $^{125}\text{I}$ -labeled insulin measured. Bound insulin was determined by eluting IR off the plate with 0.1M HCl and measuring the amount of IR eluted in a g- counter. For data analysis, non-specific binding was defined as the amount 10 bound in presence of  $10^6\text{ M}$  unlabeled insulin.

#### RESULTS

The effect of  $\text{D}^k$ -(61-85) on both substrate (poly-[E,Y]) phosphorylation and IR autophosphorylation as a function of the peptide concentration, wherein IR tyrosine kinase 15 activity is induced with  $10^{-6}\text{ M}$  insulin, was determined. Both IR phosphorylation and autophosphorylation were strongly inhibited at a concentration of  $10 \mu\text{M}$   $\text{D}^k$ -(61-85). The basal activity of IR (no insulin added) was inhibited 24-40% by  $\text{D}^k$ -(61-85) and  $\text{K}^k$ -(61-85). The effect of  $\text{K}^k$ -(61- 20 85) was significantly weaker than  $\text{D}^k$ -(61-85) on autophosphorylation, with  $\text{EC}_{50}$  values [95% confidence intervals] of  $4.0 \mu\text{M}$  [ $2.2$ - $7.2 \mu\text{M}$ ] and  $1.2 \mu\text{M}$  [ $0.3$ - $2.2 \mu\text{M}$ ] for  $\text{K}^k$ -(61-85) and  $\text{D}^k$ -d(61-85), respectively, whereas no difference was observed with respect to substrate 25 phosphorylation. None of the control peptides (e.g. ACTH-(1-24) are substrates for IR tyrosine kinase.

No significant depletion (degradation or adsorption), as examined by HPLC and  $^{125}\text{I}$ -labeled  $\text{D}^k$ -(61-85),  $\text{K}^k$ -(61-85), ACTH-(1-24), or dynorphin A was observed during the 30 experimental period at concentrations above  $0.1 \mu\text{M}$ . The  $\text{D}^k$ -(61-85) peptide does not affect IRKD phosphorylation, as demonstrated by pre-incubation of maximally autophosphorylated and  $^{32}\text{P}$ -labeled IR for 1 hr on ice with  $10 \mu\text{M}$  peptide and subsequent incubation with  $500 \mu\text{M}$  cold ATP 35 for 0-60 min at room temperature.

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The D<sup>k</sup>-(61-85) had no effect on the binding of insulin to IR. The EC<sub>50</sub> IR autophosphorylation was about  $3 \times 10^{-9}$  M insulin, corresponding approximately to K<sub>d</sub>( $2.8 \times 10^{-9}$ M). D<sup>k</sup>-(61-85) at a concentration of 10  $\mu$ M inhibits 5 autophosphorylation at all insulin concentrations.

D<sup>k</sup>-(61-85) at a concentration of 3  $\mu$ M inhibits the insulin-induced IR autophosphorylation, but not the insulin receptor kinase domain phosphorylation, when IR and IRKD are used at comparable activities. IR is not a significant 10 substrate for IRKD in the absence of insulin. IR becomes a significant substrate for IRKD when insulin is added. This observation is facilitated by the inhibitory effect of the peptide on IR autophosphorylation, because the IR phosphorylation as mediated by the tyrosine kinase of IR 15 itself and the phosphorylation mediated by IRKD would otherwise be indistinguishable.

In the next study, the uptake of glucose in rat adipocytes was performed. Adipocytes were prepared from non-starved male rat epididymal fat pads (1.2-1.6 g fat per rat) 20 by collagenase digestion. The digest was filtered (25  $\mu$ l), washed and resuspended in approximately 4 times the cell volume (estimated by lipocrit) in Krebs-Ringer's/HEPES (KRH) with 5% BSA. Only plastic tubes were used. An aliquot was removed for Coulter counting after staining with 2% osmium 25 tetroxide, filtration and dilution in saline. 50  $\mu$ l of adipocyte suspension was added to the pre-incubation mix; 300  $\mu$ l buffer, 50  $\mu$ l insulin (80 nM) or buffer; 50  $\mu$ l test solution (10 x) or buffer and incubated for 30 min at 37°C in a shaking water bath. A blank without cells was included 30 for background counting. D-[<sup>14</sup>C]-glucose was subsequently added (about 10<sup>5</sup>dpm/sample) and incubation continued for 60 min. The incubation was terminated by layering the 400  $\mu$ l sample on top of silicone oil, followed by a 30 sec. microcentrifuge spin, and cutting the adipocytes (thin layer 35 of cells on top of the oil, buffer under oil) into LS vials with scintillation fluid. Glucose concentration was about

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300 nM (specific activity = 295 mCi/mmol).

The effect of increasing concentrations of insulin in 30  $\mu\text{M}$  D<sup>k</sup>-(61-85) on glucose uptake was determined. Insulin induced maximally an 8-11 fold increase in glucose uptake as compared to basal uptake. Addition of D<sup>k</sup>-(61-85) increased the maximal uptake to about 14-18 fold of basal, a glucose uptake above maximal insulin stimulation. At low concentrations of insulin (plasma level and lower), 30  $\mu\text{M}$  D<sup>k</sup>-(61-85) increased glucose uptake is equal to or more than that induced by insulin on a molar basis.

Various fragments of D<sup>k</sup>-(61-85) were prepared by enzymatic digestion with specific peptidases: endo K, which gave fragments 60-68 and 69-85; endo E, which gave fragment 78-85; CP Y, which provided fragment 61-84; and in addition, the starting fragment was iodinated, which would be expected to occur at the terminal tyrosines. Each of the fragments were tested for biological activity after purification (greater than 95%) by HPLC and added to the cells to achieve a final concentration of 30  $\mu\text{M}$ . The results reported as percent activity of the mean  $\pm$  standard error, with the starting fragment being 100 are as follows (61-68) 19  $\pm$  22; (69-85) 87  $\pm$  2; (78-85) 15  $\pm$  3; (61-84) 19  $\pm$  3; iodinated fragment 9  $\pm$  10.

The effect of D<sup>k</sup>-(61-85) in whole rats was determined. D<sup>k</sup>-(61-85) (2.5 mg/kg) and insulin (10  $\mu\text{g}/\text{kg}$ ) on the blood glucose levels in rats (100-300 g) was determined. The peptide and insulin were injected intravenously after the animals had been anesthetized with pentobarbital. All animals were starved 16-20 hr prior to experimentation. Each determination was based on results as obtained from 42 rats, where the same rats were used in the four treatment schedules: control, peptide alone, insulin alone, and insulin plus peptide. The control showed no significant change in blood glucose over the 240 min during which determinations were made. At about 20 min after injection of peptide, the blood glucose had dropped to about 65% of

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its original value and then slowly rose back to about the original value at about 90 min and was maintained about the same level. A similar result was observed with the injection of insulin. However, where the insulin and 5 peptide were injected together, the glucose dropped within about 20 min to about 55% of its original value, slowly rose to about 85% of its original value at about 195 min, and then gradually increased to about 90% at about 240 min. Calculation of the area between the control curve and the 10 experimental curves from T = 0 to T = 240 min showed that the area for insulin plus peptide is significantly larger than that of either insulin or peptide alone, indicating a prolonged hypoglycemic period as compared to the injection of insulin or peptide alone. These results support that not 15 only does the peptide have an effect on the insulin receptor in vivo, but also that skeletal muscle, the main organ responsible for glucose transport, is affected.

Based on the above data, it may be concluded that D<sup>k</sup>-(61-85) peptide enhances cellular glucose uptake both in the 20 absence and presence of insulin. The peptide effect is increased upon stimulation with insulin. Maximal peptide effect is reached at a peptide concentration of 10-20  $\mu$ M. The peptide causes enhanced glucose uptake significantly above that induced by maximal insulin stimulation. The 25 effect in vitro is maximal after 20 min incubation of the cells with peptide. Intravenous injection of 2.5 mg/kg D<sup>k</sup>-(61-85) peptide causes a decrease in blood glucose in whole animals. It is accentuated when insulin is injected together with the peptide. In these experiments 30 the levels of serum-insulin were unchanged.

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Example 3. Class I MHC Peptide Regulation of EGF Receptor

Peptides. Peptides (Table 3) were synthesized by Applied Biosystems, Inc. (Foster City, CA). The crude D<sup>k</sup>- (61-85) peptide was purified by preparative high performance liquid chromatography (HPLC) to better than 97% homogeneity as judged by analytical HPLC monitoring of absorbance at 214 and 278 nm. The a<sub>2</sub>- and a<sub>3</sub>-derived peptides were more than 90% pure. Identity was confirmed by amino acid composition and mass spectrometry. The peptides were dissolved in 0.1M HCl and stored at 1.0 mM in 200 µl aliquots at -80°C.

Table 3. MHC Class I Derived Peptides

	<u>Peptide</u>
	<u>Sequence</u>
	D <sup>k</sup> - (61-85)      ERETQIAKGNEQSFRVDLRTLRYY
15	D <sup>b</sup> - (137-161)      DMAAQITRRKWEQSGAAEHYKAYLE
	D <sup>b</sup> - (152-176)      AAEHYKAYLEGECVEWLHRYLKNGN
	D <sup>b</sup> - (197-221)      GEVTLRCWALGFYPADITLTWQLNG

Hormones. EGF (mouse) and PDGF (human) were purchased from Collaborative Research, Inc., Bedford, Mass. TGF<sub>a</sub> (rat) was purchased from Peninsula Laboratories, Belmont, CA. <sup>125</sup>I-labeled EGF (480 Ci/mmol) was purchased from ICN Biomedicals, Inc.

Preparation of Adipocytes. The procedure has been described in detail previously. Briefly, male Wistar rats (100-150 g) were decapitated, and the epididymal fat pads removed and minced with scissors into KRHB (Krebs-Ringer/HEPES/Bovine serum albumin (BSA) buffer: 80 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl<sub>2</sub>, 1.2 mM MgSO<sub>4</sub>, 50 mM HEPES, 5% BSA (Sigma, radioimmunoassay grade), pH 7.2) containing 5 mM D-glucose and 1 mg/ml collagenase (type I, Worthington) and digested (1 hr, 37°C) with gentle shaking (250 cycles per

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minute). The adipocytes were washed five times in KRHB (each wash in a volume ten times the cell volume). The adipocyte layer was finally diluted with KRHB to a 10% (v/v) suspension as estimated by the volume of packed cells 5 (lipocrit).

Glucose uptake in vitro. The uptake of [<sup>14</sup>C]glucose (D-[U-<sup>14</sup>C]glucose, Amersham, (300 Ci/mol)) by isolated rat adipocytes was measured as follows unless otherwise stated: 10 50 µl adipocyte cell suspension was pipetted into "Nunc-Immuno tube minisorp" (Nunc, Denmark) and preincubated at 37°C with gentle shaking (225 cycles per minute) for 30 min. Hormones and peptides were added in 50 µl KRHB and incubated for 30 min at 37°C. Before addition to cells, all solutions were neutralized to pH 7.2. [<sup>14</sup>C]glucose tracer 15 ( $\approx$ 100,000 dpm) was added and the incubation continued for another 20 min at 37°C. The assay was terminated by centrifugation of 100 µl sample on top of 250 µl silicone oil (Thomas Scientific) in 500 µl tubes in a microcentrifuge (10,000 x g) for 1 min. The tube was cut just below the 20 adipocyte layer and the amount of radioactivity in the adipocytes determined by scintillation counting. The precise concentration of [<sup>14</sup>C]glucose was calculated from total counts added. Data were not corrected for trapping, which in previous experiments was found to be negligible.

25 Cell-associated <sup>125</sup>I-labeled EGF. 50 µl adipocyte cell suspension was pipetted into "Nunc-Immuno tube minisorp" (Nunc, Denmark) and preincubated at 37°C with gentle shaking (225 cycles per minute) for 30 min. The cells were incubated for 30 min at 37°C with 50 µl KRHB containing 30 600 pM <sup>125</sup>I-labeled EGF and different concentrations of non-radioactive EGF. The assay was terminated by centrifugation of 75 µl sample as described above for glucose uptake, and the cell-associated as well as the free <sup>125</sup>I-labeled EGF was determined with a g-counter. Non-specific cell-associated

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$^{125}\text{I}$ -labeled EGF was defined as cell-associated  $^{125}\text{I}$ -labeled EGF in the presence of 128 nM non-radioactive EGF. The data were calculated as specific cell-associated  $^{125}\text{I}$ -labeled EGF and the maximal value was in each experiment set to 100%.

5 RESULTS

EGF alone stimulated glucose uptake in rat adipocytes  $\approx$ 50% above the basal level. However, when D<sup>k</sup>(61-85) peptide was added at a concentration of 30  $\mu\text{M}$ , the glucose uptake was enhanced 5-6 fold above the basal uptake level. The 10 peptide did not affect the EC<sub>50</sub> for the EGF effect on glucose uptake. Maximal effect of EGF and D<sup>k</sup>(61-85) was obtained after approximately 10-20 min. The peptide was active in a concentration range of 5-30  $\mu\text{M}$  and had an EC<sub>50</sub>  $\approx$ 10-15  $\mu\text{M}$ . The maximal effect obtained with EGF and peptide alone was 15  $\approx$ 50% of the maximal effect obtained with insulin. EGF and peptide, in combination with increasing concentrations of insulin, resulted in increasing glucose uptake until the maximal level attained with insulin and peptide was reached.

Experiments with  $^{125}\text{I}$ -labeled EGF demonstrated specific 20 cell-associated EGF with an apparent affinity in the low nanomolar range, which corresponds to the affinity for EGF binding reported with adipocytes and other cell types. The presence of the peptide did not affect the binding affinity of  $^{125}\text{I}$ -labeled EGF. The total cell-associated  $^{125}\text{I}$ -labeled 25 EGF was unaffected by the presence of peptide 119  $\pm$ 12 (mean  $\pm$  S.E.M.; no peptide = 100; n=3). Peptide also did not affect the amount of non-specific cell-associated  $^{125}\text{I}$ -labeled EGF (57  $\pm$  8 with peptide versus 53  $\pm$  12 without peptide).

30 Incubation of adipocytes with 50 nM TGF<sub>a</sub> increased [<sup>14</sup>C]glucose uptake  $\approx$ 50% above basal, whereas 50 nM TGF<sub>a</sub> in combination with 30  $\mu\text{M}$  D<sup>k</sup>(61-85) increased [<sup>14</sup>C]glucose uptake  $\approx$ 5 fold above basal. This effect of TGF<sub>a</sub> alone or in 35 combination with D<sup>k</sup>(61-85) on [<sup>14</sup>C]glucose uptake is thus similar to the effect found for EGF.

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Platelet derived growth factor (PDGF) alone or together with D<sup>k</sup>(61-85) had no effect on glucose uptake in adipocytes.

Three other peptides derived from the MHC class I molecule were also tested for their effect on glucose uptake when combined with EGF. D<sup>b</sup>(137-161) and D<sup>b</sup>(152-176), derived from the a<sub>2</sub> region, and D<sup>b</sup>(197-221), derived from the a<sub>3</sub> region, had no or only a small effect on glucose uptake compared to the D<sup>k</sup>(61-85) peptide.

10 Example 4. Amino Acid Residues of MHC Class I Peptide Essential for Biological Activity

Materials and Methods

Glucose Transport in Adipose Cells. The biological activity of the peptides was measured by their effect on glucose uptake in rat adipose cells as described (Stagsted, et al. (1991) J. Biol. Chem. 266:12844-12847). Briefly, rat adipose cells were obtained from epididymal fat pads and suspended in Krebs-Ringer HEPES buffer (KRH) with 5% bovine serum albumin at a lipocrit of 10% (final). The peptide effect was measured in cells maximally stimulated with insulin (8 nM). After equilibration at 37°C for 30 min the cells were incubated for 30 min at 37°C with buffer (basal), 8 nM insulin plus peptide. <sup>14</sup>C-D-glucose was added, and the cells were incubated for an additional 30 min and harvested on oil. Biological activity was measured by a dose-response curve to interpolate the EC<sub>50</sub> value, taking the maximum enhancement of insulin effect (about 40% over the insulin-only maximum) as 100%. Most of the peptides were not tested at higher concentrations than 30 μM. Peptides that enhanced the maximum insulin effect by less than 20% at 30 μM were considered inactive. Accordingly, three categories of peptides were defined: Full activity, EC<sub>50</sub><10 μM; reduced activity, 10 μM≤EC<sub>50</sub>≤30 μM; no activity, EC<sub>50</sub>>30 μM.

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Peptides. The peptides were assembled stepwise either on a phenylacetamidomethyl (PAM) resin using the t-Boc NMP/HOBt protocol of an Applied Biosystems Model 430A peptide synthesizer, or on a p-alkoxy benzyl alcohol (Wang) resin using a modified Fmoc/BOP protocol of a Milligen/Bioscience Model 9600 synthesizer. The side chain protecting groups were as follows: for t-Box chemistry, Arg(MTS), Asp(OChx), Glu(OBzl), Lys(Cl-Z), Ser(Bzl), Thr(Bzl), and Tyr(Br-Z); for Fmoc chemistry, Arg(Pmc), 5 Asp(OtBu), Glu(OtBu), Lys(t-Boc), Ser(tBu), Thr(tBu) and Tyr(tBu). The t-Boc-assembled peptides were deprotected/cleaved from the solid support using HF in the presence of anisole, ethanedithiol, and dimethylsulfide as scavengers. After conversion of the hydrofluoride to the 10 acetate salt by ion-exchange column chromatography, the peptides were purified to greater than 98% homogeneity by preparative HPLC using a Vydac C18 (2.2x25 cm) column and appropriate linear gradients of 0.1% TFA-buffered acetonitrile in 0.1% aqueous TFA. The Fmoc-assembled 15 peptides were deprotected/cleaved from the resin using TFA in the presence of thioanisole, ethanedithiol, water and phenol as scavengers, and purified by preparative high performance liquid chromatography as described above. The desired peptides were confirmed by sequence analysis, amino 20 acid composition, and fast atom bombardment mass spectrometry. The peptides were activated by incubation of 25 1 mM stock solution at 37°C in 0.1 M NaCl overnight (Stagsted, et al. (1991) J. Biol. Chem. 266:12844-12847).

Circular Dichroism (CD). CD spectra were recorded on 30 a JASCO J-600 calibrated against d-camphorsulfonic acid using  $\Delta E$  (290.5 nm) = +2.38 M<sup>-1</sup> cm<sup>-1</sup>. Rectangular cuvettes with path lengths of 0.01 cm were used for recording spectra of 1 mM peptide stock solutions.

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Aggregation. Peptide stock solution was diluted in KRH buffer to 30  $\mu$ M, incubated (30 min, 37°C), then centrifuged at 12,000 g for 10 min. The amount of peptide remaining in solution was measured spectrophotometrically by absorbance 5 at 278 nm ( $E$  1200 M<sup>-1</sup> cm<sup>-1</sup> per tyrosine residue).

#### RESULTS

Biological Activity. The D<sup>k</sup>-(62-85) and D<sup>k</sup>-(69-85) peptides were analyzed by alanine scan (Cunningham and Wells 1989) Science 244:1081-1085; Cunningham and Wells (1991) 10 Proc Natl Acad Sci (USA) 88:3407-3411; Wells (1991) Methods Enzymology 202:390-411) which involves systematic replacement of residues with alanine to assess the importance of each residue for biological activity. Table 4 presents the potency of D<sup>k</sup>-(62-85) and D<sup>k</sup>-(69-85), of 13 15 peptides in the alanine scan, and of five additional peptides of interest. Figure 1 summarizes the relative importance of each residue in the peptide with respect to biological activity and ordered structure as well as the position of the residues on the  $\alpha$ -helix. Substitution of 20 Phe<sup>74</sup> (d), Leu<sup>81</sup> (f), and Leu<sup>82</sup> (g), all resulted in loss of activity. Substitution of Ala for Phe<sup>74</sup> produced a peptide which was completely inactive even at 90  $\mu$ m, the highest concentration tested. Peptides with alanine instead of Leu<sup>78</sup> 25 (e), Arg<sup>83</sup> (h), or Tyr<sup>84</sup> (i) all had reduced activity compared to D<sup>k</sup>-(69-85) (b). Replacement of Glu<sup>71</sup> (c) or Tyr<sup>85</sup> (j) yielded peptides that were even more potent than the original.

The alanine scan with double residue changes showed (in m) that neither Asn<sup>70</sup> nor Asp<sup>77</sup> are important for activity. 30 Peptide 1 had reduced activity, but the data did not allow for a determination as to whether Gly<sup>69</sup> or Val<sup>76</sup> is most important. The three other peptides with double alanine substitutions (k, n, o) were all inactive. In peptide k the essential residue is likely to have been Arg<sup>75</sup>, as residues 35 62-68 can be deleted entirely (cf. a, b) without loss of

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activity. The data did not allow for a determination as to whether the inactivity of peptides n and o were due to substitution of only one or both of the residues. However, as the chimeric peptide r, with Gly<sup>79</sup>, was moderately active, 5 Gln<sup>72</sup> seems more responsible than Arg<sup>79</sup> for the loss of activity in peptide n.

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Table 4. Sequence, Biological Activity, and Aggregation of MHC Class I-Derived Peptides.

PEPTIDE	SEQUENCE	RES. SUBS.	EC50 <sup>a</sup> (μM)	Aggregation <sup>b</sup>
D <sup>k</sup> - (62-85)	RETQIAKGNEQSFRVDLRTLLRYY		2	70±2
D <sup>k</sup> - (69-85)	GNEQSFRVDLRTLLRYY		5	NTC
SINGLE SUBSTITUTIONS:				
[Ala 71]-D <sup>k</sup> - (69-85)	GNAQSFRVDLRTLLRYY	E	3	50±10
[Ala 74]-D <sup>k</sup> - (69-85)	RETQIAKGNEQSARVDLRTLLRYY	F	>90	33±4
[Ala 78]-D <sup>k</sup> - (69-85)	GNEQSFRVDARTLRLYY	L	2	39±6
[Ala 81]-D <sup>k</sup> - (69-85)	GNEQSFRVDLRTLALRYY	L	=3	64±6
[Ala 82]-D <sup>k</sup> - (69-85)	GNEQSFRVDLRTLARYY	L	=50	35±4
[Ala 83]-D <sup>k</sup> - (69-85)	GNEQSFRVDLRTLAYY	R	=20	91±1
[Ala 84]-D <sup>k</sup> - (69-85)	GNEQSFRVDLRTLLRAY	Y	=15	89±2
[Ala 85]-D <sup>k</sup> - (69-85)	GNEQSFRVDLRTLLRYA	Y	1	88±5
DOUBLE SUBSTITUTIONS:				
[Ala 68, 75]-D <sup>k</sup> - (62-85)	RETQIAAGNEQSFAVDLRTLLRYY	K, R	>60	44±6
[Ala 69, 76]-D <sup>k</sup> - (62-85)	RETQIAKANEQSFRADLRTLLRYY	G, V	=30	42±4
[Ala 70, 77]-D <sup>k</sup> - (62-85)	RETQIAKGAEQSFRVALRTLLRYY	N, D	7	72±4
[Ala 72, 79]-D <sup>k</sup> - (62-85)	RETQIAKGNEASFRVDLATLRLYY	Q, R	=90	54±4
[Ala 73, 80]-D <sup>k</sup> - (62-85)	RETQIAKGNEQAFAFRVDLALLRYY	S, T	>90	59±4
OTHER PEPTIDES:				
HLA-A2- (69-85)	AHSQTHRVDLGLTGLRGYY		Inactive	NT
HLA-A2- (69-76)D <sup>k</sup> - (77-85)	AHSQTHRVDLRTLLRYY		Inactive	NT
D <sup>k</sup> - (69-76)HLA-A2- (7-85)	GNEQSFRVDLGLTGLRGYY		Active	NT
HLA-B27- (69-85)	AKAQTDREDLRTLLRYY		Inactive	NT
(Phe 74)-HLA-B27- (69-85)	AKAQTFREDLRTLLRYY		Active	NT

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Legend to Table 4

<sup>a</sup>EC<sub>50</sub> value for glucose uptake as measured in the rat adipose cell assay.

<sup>b</sup>Aggregation measured by centrifugation of 30 μM peptide solution in KRH for 10 min at 12,000 g and the amount of peptide remaining in solution determined spectrophotometrically. The numbers indicated are per cent precipitated and are mean ± SEM of 3 experiments.

<sup>c</sup>NT, not tested.

10 <sup>d</sup>Maximal peptide effect in cells fully stimulated by insulin was 40% enhancement over insulin alone. Peptides giving less than 20% enhancement at 30 μM were considered inactive (EC<sub>50</sub>>30 μM).

The essential role of Phe<sup>74</sup> is shown not only by the  
15 inactivity of d, as well as p and q (which contain many of the other residues shown to be essential), but most dramatically by the fact that the inactive human peptide s became fully active on changing Asp<sup>74</sup> to Phe<sup>74</sup> in peptide t.

Circular Dichroism (CD). Residues identified in the  
20 alanine scan to be important for biological activity were examined for their role in the maintenance of an ordered structure. Measurements at 1 mM in 0.1 M NaCl yielded a variety of complex CD spectra, so that simple classification into recognized structures (such as α-helix; 9, 10) was  
25 often not possible. Peptide j, which is fully active, has a spectrum with maxima at 205 nm (negative) and 195 nm (positive), suggesting a high content of ordered structure. The original unsubstituted peptides a and b as well as the two other most active peptides (c, t) have a similar  
30 positive CD signal at 195 nm. Peptides h and k, with reduced activity and no activity, respectively, have CD spectra with both a positive and negative maximum, but without the typical profile of peptide j. Peptides (e, i, r), all of which have reduced activity, and the inactive

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peptides (f, n, o, s) also fall into this category. The CD spectrum of peptide m is also in this category, although its activity is comparable to that of peptide b. Peptides g and q (no activity) and peptide 1 (reduced activity) have spectra with a negative maximum, no positive maximum, but with an indication of some molecules with ordered structure. Only the inactive peptides d and p have the typical spectrum of a random coil, with a negative maximum at 195 nm. The degree of ordered structure as estimated by CD and the biological activity ( $c^2 = 10.6$ ;  $P < 0.05$ ) are positively correlated as determined, by a conservative test ignoring the rank order of categories in the  $3 \times 3$  contingency table.

Aggregation. Table 4 shows the extent of aggregation of the various peptides. Using scatter plot diagram analysis it is apparent that a positive correlation ( $r=0.56$ ,  $P < 0.05$ ) exists between the biological activity of a peptide and the ability of the peptide to self-interact and form aggregates. Peptide self-interaction (aggregation) was also found to be correlated with the degree of ordered structure ( $r=0.49$ ,  $P < 0.05$ ).

#### Effect of [Ala<sup>85</sup>]-D<sup>k</sup>-(69-85) Peptide In Vivo

To examine the effect of peptide on blood glucose level in vivo, 3.0 mg/kg of the [Ala<sup>85</sup>]-D<sup>k</sup>-(69-85) peptide in 0.3 ml KRH buffer was injected subcutaneously into rats. In a parallel group of animals, 0.3 ml KRH buffer was injected subcutaneously as a negative control. Blood glucose levels were determined prior to injection and then for 330 m at 30 m intervals after injection. Values at each time point were determined from the average blood glucose level of either 6 (KRH only) or 7 (peptide) rats. The blood glucose level of rats treated with buffer alone was not significantly affected during the time period examined. However, within 30 m blood glucose levels in rats treated with peptide dropped to approximately 70% of blood glucose levels prior

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to treatment and dropped as low as approximately 60% within the time period examined. Even after 330 m, blood glucose remained at significantly decreased levels (approximately 60% of blood glucose prior to treatment). These data 5 therefore support an in vivo role for peptide-mediated modulation of surface expression of receptors.

SUMMARY

The data summarized in Figure 1 show that the biologically active peptides must be capable of assuming an 10 ordered structure. However, not all peptides with ordered structure are active. Thus ordered structure is necessary but not sufficient for biological activity. The systematic alanine substitutions show that residues required for full 15 biological activity upon substitution with alanine do not necessarily affect the ordered structure. This supports the theory that the residues exclusively required for biological activity are part of the site on the peptide that interacts with a binding site in the cell membrane. Figures 1B and 1C further illustrate that most of the residues required for 20 biological activity are localized on the hydrophilic (cationic) side of the peptide, assuming an  $\alpha$ -helical structure, whereas other residues form the hydrophobic side of the helix. It should also be noted that the information obtained from the alanine scan is premised on the assumption 25 that the function of each residue is independent of the other residues in the peptide.

The CD studies imply that peptides in ordered structure may form an  $\alpha$ -helix or perhaps to some extent a tighter  $3_{10}$  helix for the [Ala<sup>85</sup>]-D<sup>k</sup>-(69-85) peptide. Only one residue 30 (Phe<sup>74</sup>) seems essential for the ordered structure and thus helix formation. The significance of Phe<sup>74</sup> is particularly striking as its substitution with alanine not only resulted in loss of biological activity in the mouse peptide, but introduction of Phe<sup>74</sup> into a human sequence resulted in 35 biological activity of an otherwise inactive peptide.

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Most of the active peptides aggregate at high concentration, implying a self-interaction between peptide molecules. The helical wheel and rod of the D<sup>k</sup>-(69-85) peptide in Figures 1B and 1C illustrate that the hydrophilic 5 (cationic) residues are localized on one side and the hydrophobic residues on the opposite side of the  $\alpha$ -helix. This distribution may promote intermolecular interactions, which could be of significance in the interaction between the peptides and cells. Thus, the ability of biologically 10 active peptides to exhibit self-peptide intermolecular interactions supports the theory that peptides bind to the  $\alpha_1$ -helix of the native MHC Class I molecules on the cell surface. Such binding of peptides to the MHC Class I  $\alpha_1$ -helix would be analogous to self-peptide interactions since 15 a portion of the MHC Class I  $\alpha_1$ -helix is homologous to the peptide. Furthermore, native MHC Class I molecules may interact with one another to form dimers, tetramers, and perhaps even larger oligomers in the membrane (Chakrabarti, et al. (1992) Biochemistry 31:7182-7189; Krishna, et al. 20 (1992) Nature 357:164-167).

Search of the protein sequence database showed that all MHC Class I molecules have sequence similarity greater than 70% to D<sup>k</sup>-(61-85), whereas the highest similarity of a protein other than MHC Class I was only 32%. The degree of 25 phylogenetic conservation of an amino acid residue in the MHC Class I molecule for the D<sup>k</sup>-(69-85) molecule is unrelated to the importance of the residue for biological activity. However, this is not surprising as conservation of a given amino acid in different MHC Class I molecules may 30 simply reflect a critical role of three-dimensional structure. Here, the biological activity is related to inhibition of receptor internalization. The present data support that peptide activity may depend upon a direct binding of the peptide to the  $\alpha_1$  domain of MHC Class I on 35 the cell surface. Such an interaction is analogous to the peptide-peptide self-interaction.

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Example 5. MHC Class I Peptide Inhibition of Internalization of Glucose Transporters and IGF-II Receptors

Materials and Methods

Glucose Transport Activity, Photoaffinity Labeling, and

5 Immunoblotting. Briefly, adipose cells were equilibrated at 37°C for 30 min and then incubated for an additional 30 min at 37°C with buffer (basal), 10 μM D<sup>k</sup>(62- 85), 150 nM insulin (which induces maximal stimulation of both glucose transporter and IGF-II receptor translocation to the cell surface (Appell, et al. (1988) *J. Biol. Chem.* 263:10824-10829), or 150 nM insulin plus 10 μM D<sup>k</sup>(62-85). 3-O- methylglucose transport was measured according to the method of Karnieli, et al. (1981) *J. Biol. Chem.* 256:4772-4777. Photoaffinity labeling was carried out by mixing 15 2.5 ml of cell suspension with 500 μCi 2-N-4(1-azi-2,2,2-trifluoroethyl)benzoyl-1,3-bis(D-mannos -4-yloxy)-2-propylamine (ATB-[2-<sup>3</sup>H]-BMPA) and immediately exposing the cells to UV irradiation for 3 x 1 min. The cells were then washed and solubilized in phosphate-buffered saline 20 containing 2% Thesit (Boehringer Mannheim), 5 mM EDTA, 2 mM N-ethylmaleimide, 1 mM phenylmethylsulfonyl- fluoride, 1 μg/ml each of pepstatin, leupeptin, aprotinin, and α2-macroglobulin, and the solubilized material was immunoprecipitated first with anti-GLUT4 antiserum and 25 protein-A agarose (21), and subsequently with anti-GLUT1 antiserum. Polyclonal antisera to the synthetic peptide antigens (provided by Hoffman-La Roche) was raised in rabbits. The labeled proteins in the precipitates were separated by 10% SDS-PAGE, each lane was cut into 3 mm 30 slices, and the slices were processed for scintillation counting.

For immunoblotting, 5 ml of cells were used for preparation of plasma membrane (PM) and low-density microsomes (LDM) as described (Simpson, et al. (1983) 35 *Biochim. Biophys. Acta.* 763:393-407). Recovery of the

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membrane fractions was determined by measuring protein (bicinchoninic acid assay, Sigma), and 50 µg of protein were run on 10% SDS-PAGE, transferred to nitrocellulose, and immunoblotted with antisera against GLUT1, GLUT4, or IGF-II receptors (the latter kindly provided by Dr. Robert C. Baxter, University of Sydney, Australia) followed by <sup>125</sup>I-labeled protein-A. The radioactive bands on the nitrocellulose were excised and counted in a g-counter. Control experiments using SDS-PAGE analysis of proteins from subcellular fractionation of adipose cells treated with buffer, peptide, insulin, or insulin plus peptide show that the peptide does not affect the overall protein distribution within the various subcellular fractions.

Cross-linking and Binding of IGF-II. For cross-linking of <sup>125</sup>I-IGF-II, cells were incubated with or without D<sup>k</sup>(62-85) peptide and with or without insulin as described above, followed by a 30-min incubation with 250 pM <sup>125</sup>I-labeled IGF-II (2,000 Ci/mmol, Amersham) in the absence or presence of 100 nM unlabeled IGF-II. Cells were isolated on oil and transferred to albumin-free incubation buffer containing 1 mM disuccinimidyl suberate (Pierce) and incubated for 10 min at 16°C. The cells were solubilized in sample buffer and run on 10% SDS-PAGE. The IGF-II receptor was identified by autoradiography and quantitated by counting the excised bands.

For competition binding curves, cells were preincubated as described and then incubated for an additional 30 min with 8 nM insulin or 8 nM insulin plus 10 µM D<sup>k</sup>(62-85) peptide. KCN was added to a final concentration of 2 mM and the incubation was continued for another 15 min. Subsequently, the cells were washed and resuspended in buffer with 2 mM KCN; 50 µl of cells were mixed with 50 µl of <sup>125</sup>I-labeled IGF-II (final concentration 50 pM) in the absence or presence of various concentrations of non-radioactive IGF-II (Sigma) and further incubated for

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30 min at 37°C before harvesting the cells on oil. Acid wash of cells incubated with  $^{125}\text{I}$ -IGF-II in the presence or absence of KCN showed that KCN effectively blocks 85-90% of receptor internalization. Thus, whole cell binding in the 5 presence of KCN reflects surface-bound IGF-II.

Kinetics of Internalization of GLUT4 and IGF-II Receptors. Adipose cells were preincubated as described and incubated an additional 30 min at 37°C with 8 nM insulin in 10 the absence or presence of 20  $\mu\text{M}$  [Ala<sup>85</sup>]-D<sup>k</sup>(69-85). For GLUT4 experiments, 4.0 ml volumes of cells were then photolabeled with 750  $\mu\text{Ci}$  ATB-[<sup>3</sup>H]-BMPA as described above except that UV irradiation was limited to 2  $\times$  30 s and the cells were further protected from radiation damage by 15 covering the samples with plastic lids. Photolabeled cells were subsequently incubated for an additional 0, 10, 20, 30, or 60 min. At each time point, samples were taken for measurement of glucose transport activity, KCN was added to the remaining cells to a final concentration of 2 mM to 20 arrest subcellular trafficking, the cells were harvested, and PM and LDM were prepared. The amounts of radiolabeled GLUT4 in PM and LDM were determined by immunoprecipitation as described above. For IGF-II receptors,  $^{125}\text{I}$ -IGF-II was added to 50 pM to insulin- or insulin plus 25 peptide-stimulated cells in the presence or absence of 50 nM unlabeled IGF-II in a final volume of 100  $\mu\text{l}$  and the cells were harvested after 5, 10, 15, 20, 30, or 60 min incubation. Upon harvest, the cells were transferred to 100  $\mu\text{l}$  buffer at either pH 7.2 or pH 2.0 (final pH 3.0), 30 incubated on ice for 5 min, and harvested by centrifugation on oil. Internalized IGF-II was defined as specific acid resistant  $^{125}\text{I}$ .

Peptides. D<sup>k</sup>(61-85) (ERETQIAKGNEQSFRVDLRTLRYY) was used in previous studies. However, in the present study an 35 N-truncated version of the peptide, D<sup>k</sup>(62-85), with the same

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potency was used because the N-terminal Glu<sup>61</sup> residue of D<sup>k</sup>-(61-85) is a potential source of peptide heterogeneity due to possible cyclization to poly-glutamic acid (pGlu). For kinetic studies, [Ala<sup>85</sup>]-D<sup>k</sup>-(69-85) was used instead of D<sup>k</sup>-(62-85) because it is as potent as D<sup>k</sup>-(62-85) but poses fewer technical difficulties in handling. Synthesis, purification, and methods to assure the correct identity of the peptides were performed as described above. The D<sup>k</sup>-(62-85) peptide used in the present study was maintained in an active conformation by incubation of 1 mM stock solution at 37°C in 0.1 M NaCl overnight, whereas [Ala<sup>85</sup>]-D<sup>k</sup>-(69-85) was used directly from a 1 mM stock solution in water.

#### RESULTS

Glucose Transport Activity and Photolabeling with ATB-BMPA. Table 5 shows that the D<sup>k</sup>-(62-85) peptide enhances 3-O-methylglucose transport by about 2-fold in the absence of insulin in rat adipose cells, corresponding to an increment of 0.15 fmol/cell/min. In insulin-stimulated cells, peptide also enhances glucose transport activity by about 2-fold, but with a much larger absolute effect (4.0 fmol/cell/min). Further, photolabeling with ATB-[2-<sup>3</sup>H]-BMPA shows that insulin stimulation increases cell surface GLUT4 by 27-fold compared to basal whereas D<sup>k</sup>-(62-85) plus insulin results in a 42-fold increase. Thus, the D<sup>k</sup>-(62-85) effect on enhancement of glucose transport activity corresponds well to the increase in GLUT1 in either the absence or presence of insulin.

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Table 5

	EXPERIMENTAL GROUPS				
	Basal	D <sup>k</sup> - (62-85)	Insulin	Insulin+D <sup>k</sup> - (62-85)	
5	3-O-methylglucose transport (fmol per cell per min)	0.11 ± 0.03	0.26 ± 0.05	4.1 ± 0.3	<u>8.1 ± 0.7</u>
10	ATB-BMPA cross-linking ( <sup>3</sup> H dpm per $10^6$ cells)	GLUT1 0.10 ± 0.03 GLUT4 0.17 ± 0.02	0.12 ± 0.01 0.35 ± 0.1	0.32 ± 0.05 4.7 ± 0.4	0.36 ± 0.05 <u>7.2 ± 0.5</u>

IGF-II Binding and Cell Surface IGF-II Receptors.

Table 6 illustrates that D<sup>k</sup>- (62-85) augments cell surface IGF-II binding 5-6-fold in rat adipose cells both in the absence and presence of insulin, as identified by cross-linking of <sup>125</sup>I-IGF-II. Further, the isotherm and competition binding curves in Figure 2 demonstrate that the increase in the binding of IGF-II is due to a doubling of the number of IGF-II receptors on the cell surface and an apparent change in the affinity of IGF-II receptors for ligand binding from 419±71 pM in insulin-treated cells to 145±20 pM in cells treated with insulin plus peptide (means ± standard error of 7 experiments; P<0.05; Student t-test).

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Table 6

	EXPERIMENTAL GROUPS				
	Basal	D <sup>k</sup> (62-85)	Insulin	Insulin + D <sup>k</sup> (62-85)	
5	IGF-II cross-linking (fmol per 10 <sup>6</sup> cells)	0.7 ± 0.2	<u>3.5 ± 0.6</u>	1.7 ± 0.4	<u>11.7 ± 2.9</u>

Immunoblotting of Glucose Transporters and IGF-II Receptors in Subcellular Membrane Fractions. In contrast to the GLUT4 photolabeling results, analysis of the subcellular distribution of GLUT4 by immunoblotting did not reveal any detectable effect of D<sup>k</sup>(62-85) in the absence of insulin (Table 7). In the presence of insulin, the peptide enhanced the insulin-stimulated depletion of GLUT4 in LDM, but a corresponding increase of GLUT4 in PM is not observed. Subsequent studies on the subcellular distribution of photolabeled GLUT4, i.e. cell surface GLUT4, compared to that of immunodetectable GLUT4, indicated that the peptide-mediated increase of GLUT4 on the cell surface in intact cells was accompanied by an increase in GLUT4 associated with other subcellular fractions, in particular the fat cake and nuclear membranes. This is likely to explain the lack of an immunodetectable increase in GLUT4 in PM. Similarly, immunoblotting with an IGF-II receptor-specific antiserum demonstrated a reduction in the microsomal concentration of IGF-II receptors in cells treated with insulin plus peptide compared to insulin alone. However, again a corresponding increase in IGF-II receptors in PM is not observed.

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Table 7

		EXPERIMENTAL GROUPS			
		Basal	D <sup>k-</sup> (62-85)	In- sulin	Insulin+D <sup>k-</sup> (62-85)
5	GLUT1	PM	2.6 ± 0.1	3.0 ± 0.1	5.6 ± 0.2
		LDM	5.1 ± 0.4	4.8 ± 0.6	1.3 ± 0.1
		PM	2.6 ± 0.1	3.0 ± 0.1	5.8 ± 0.1 1.0 ± 0.1
		LDM	5.1 ± 0.4	4.8 ± 0.6	1.3 ± 0.1
	GLUT4	PM	0.2 ± 0.1	0.3 ± 0.1	1.2 ± 0.1
		LDM	1.7 ± 0.2	1.8 ± 0.4	1.0 ± 0.3
		PM	0.2 ± 0.0	0.3 ± 0.4	1.3 ± 0.2 0.6 ± 0.1
		LDM	1.7 ± 0.2	1.8 ± 0.4	1.0 ± 0.3
10	IGF-II Receptor	PM	0.3 ± 0.0	0.4; 0.4	0.5; 0.5
		LDM	2.1 ± 0.1	2.0 ± 0.2	2.0 ± 0.1
		PM	0.3 ± 0.0	0.4; 0.4	0.7 ± 0.1 1.3 ± 0.1
		LDM	2.1 ± 0.1	2.0 ± 0.2	2.0 ± 0.1

It has previously been described that the insulin-induced enhancement of the number of GLUT4 in the plasma membrane appears lower when measured with immunoblotting as compared to photolabeling with ATB-BMPA (Holman, et al. 1990) J. Biol. Chem. 265:18172-18179). Indeed the increase in GLUT4 in the plasma membrane induced by insulin plus peptide is 42-fold as measured by fluorescent labeling, whereas the corresponding immunoblotting data only suggest a 2.3-fold increase, i.e. again the latter is only 14% of

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the former. Part of the reason for the lower effect as measured by immunoblotting may be that the subcellular fractionation procedure results in cross-contamination of PM with LDM, thus inflating the levels of GLUT4 and IGF-II receptors in PM in the basal state and reducing the apparent fold-response to insulin.

Kinetics of GLUT4 and IGF-II Receptor Internalization.

The  $[\text{Ala}^{85}]$ -D<sup>k</sup>(69-85) peptide completely inhibited the appearance of photolabeled GLUT4 in LDM as compared to cells treated with insulin alone. The rate of internalization of GLUT4 in cells stimulated with insulin is  $15\pm4\%$  per hr of cell surface radiolabeled GLUT4 (calculated from 6 time points in two independent experiments) and significantly ( $P<0.05$ ) reduced to  $0\pm4\%$  per hr in insulin plus peptide-treated cells (Figure 3).  $[\text{Ala}^{85}]$ -D<sup>k</sup>(69-85) also had a marked inhibitory effect (about 80%) on the rate of internalization of IGF-II receptors in insulin-stimulated cells (Figure 4)

Example 6. MHC Class I Peptide Regulation of IGF-I Receptor

20 Materials and Methods

Cell line. CHO cells over-expressing human IGF-I receptor (approximately  $2 \times 10^5$  receptors per cell) were generated by transfecting the cells with the expression vector RLDN containing the full length IGF-I receptor cDNA. The transfected cells were selected with 400  $\mu\text{g}/\text{ml}$  G418, and the cell line overexpressing IGF-I receptors was identified by a standard IGF-I ligand binding assay. The cells were grown in Ham's F12 medium supplemented with 10% fetal calf serum.

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Ligand internalization. Sub-confluent cells in 35 mm wells were used.. The monolayer was washed twice with phosphate buffered saline (PBS) and once with KRP-Hepes binding buffer (pH 7.5) at room temperature. The cells were 5 incubated in 1 ml of binding buffer containing 1  $\mu$ g/ml  $^{125}$ I-IGF-I at 37°C for 20 min. At the end of the incubation, the cells were washed 3 times with cold PBS and the surface-bound ligand was extracted with acidic binding buffer (pH 3.5) for 5 min at 4°C. After acid extraction, 10 the cell associated radioactivity was determined by lysing the cells with 0.4N NaOH and used as a measure of internalized ligand. The percent of ligand internalization was calculated by dividing the internalized cpm by surface bound plus internalized cpm. In each experiment, 500 ng/ml 15 of des-IGF-I was added to separate wells to determine the amount of ligand bound to non-receptor sites and was subtracted from the surface or internalized cpm. Des-IGF-1 is an analog of IGF-1 with the three amino acids at the N-terminal deleted. As a result, Des-IGF-1 binds normally 20 to IGF-1 receptors, but binds to IGF binding proteins with a very low affinity.

#### RESULTS

Ligand internalization. When cells are incubated with  $^{125}$ I-IGF-1, in the presence or absence of 30  $\mu$ M D<sup>k</sup>(62-85), 25 and ligand internalization measured by the acid extraction technique, the peptide leads to a marked decrease in internalized  $^{125}$ I-IGF-1 with a corresponding increase in surface bound hormone (Figure 5). When the percent  $^{125}$ I-IGF-1 internalized was calculated, D<sup>k</sup>(62-85) resulted in a 43.9  $\pm$  30 1.7% decrease ( $P < 0.01$ ) in ligand internalization. This inhibitory effect was dose dependent with half maximal effects at 8  $\mu$ M and maximal inhibition of internalization at 30  $\mu$ M.

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Example 7. Inhibition of Internalization of Transferrin Receptor

Materials and Methods

Rat adipocytes (100 ml at 31% lipocrit) were incubated  
 5 in KRHB (5% BSA) in minisorp tubes at 37°C for 30 min. In test samples, activated D<sup>k</sup>[62-85] peptide was added to a final concentration of 30 mM. Incubation of both test samples containing peptide, as well as control samples without peptide, was continued for 60 min at 37°C. <sup>125</sup>I-  
 10 transferrin (approximately 25,000 cpm/tube) was added to 2 nM with or without 200 nM unlabeled competitor. Incubation at 37 °C continued for 10 min. The cells were diluted twofold with cold KRHB (5% BSA) at pH 7.4 or pH 1.8 and held once for 10 min. Finally, the cells were separated from the  
 15 supernatant by centrifugation through oil and the number of specific counts determined using a gamma scintillation counter. Data are represented as the mean and standard error among three replicates and present as 10 minute counts (Table 8).

20 Table 8. Specific Binding and Internalization of <sup>125</sup>I-Transferrin to Rat Adipocytes

Specific Counts				
D <sup>k</sup> [62-85] Peptide	Total	Internalized	Percent Internalized	Fold Increase In Extracellular, Bound Transferrin with Peptide
-	649±21	269±36	41.1	1.86
25      +	1204±53	131±25	10.6	

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Using the protocol described above, the effect of the D<sup>k</sup>[62-85] peptide on the binding of labeled transferrin to rat adipocytes was determined as a function of peptide concentration. The results are expressed as the fold difference between the amount of transferrin bound in the presence of peptide over the amount of transferrin bound in the absence of peptide. As can be seen in Figure 6, the amount of bound transferrin increases with an increase in the concentration of peptide added up to an approximate concentration of 10 mM peptide.

For experiments using human fibroblasts, approximately 10<sup>5</sup> human fibroblasts were plated in wells of 24 well plates. Before performing the binding assay, the media was removed and replaced with KRHB (1% BSA). Plates were incubated at 37°C for 1 hr. Activated peptide at 30 mM was added where indicated and incubation proceeded for another 1 hr period at 37°C. Finally <sup>125</sup>I-transferrin was added (approximately 25,000 cpm/well) at a concentration of 2 nM, with or without 1 mM unlabeled competitor. Incubation at 37°C continued for 15 min. The labeling solution was removed and replaced with an excess of ice-cold KRHB (1% BSA) at pH 7.4 or pH 3.7. The plates were then held on ice for 5 min. The cells were solubilized with 0.1 N NaOH and counted. Data are represented as the mean and standard error among three replicates and presented as 1 minute counts (Table 9).

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Table 9. Specific Binding and Internalization of  $^{125}\text{I}$ -Transferrin to Human Fibroblasts

Specific Counts				
D <sup>k</sup> [62-85] Peptide	Total	Internalized	Percent Internalized	Fold Increase In Extracellular, Bound Transferrin with Peptide
5 -	179±26	107±39	59.8	1.97
5 +	353±19	106±12	30.0	

Using the same assay protocol as described above for rat adipocytes, the extent of surface binding of  $^{125}\text{I}$ -labeled transferrin to human fibroblasts was determined in either 10 the presence or absence of 30 mM as a function of transferrin concentration. The results are expressed as a percent bound of total added transferrin. In the absence of the peptide, transferrin binds with an EC<sub>50</sub> of 18.1 nM. In contrast, in the presence of 30 mM peptide, transferrin 15 binds with an EC<sub>50</sub> of 17.2 nM. Therefore, the peptide has no affect on the binding affinity of transferrin to its receptor.

Example 8. MHC Class I Peptide Regulation of Low-Density Lipoprotein (LDL) Receptor

20 The effect of the [Ala<sup>85</sup>]D<sup>k</sup>(69-85) MHC Class I peptide on the low-density lipoprotein (LDL) receptor in human fibroblasts was examined. Human foreskin fibroblasts were seeded in 24-well tissue culture plates with 10,000 cells

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per well and incubated in RPMI-1640 medium containing 10% FCS overnight. The cells were then washed in KRH and incubated for 1 hr in KRH containing 5% BSA in the absence or presence of 10 mM of [Ala<sup>85</sup>]D<sup>k</sup>(69-85) peptide. After 5 incubation, the wells were washed with ice-cold KRH, anti-LDL antibody added and the samples incubated at room temperature for 1 hr. The plates were then again washed, <sup>125</sup>I-protein A added in KRH containing 5% BSA. Following 10 incubation for 1 hr at room temperature, unbound <sup>125</sup>I-protein A was removed with 3 washes with KRH, the cells harvested by trypsinization for 15 min at room temperature and the amount 15 of bound <sup>125</sup>I determined in a gamma counter. Data from these experiments, each performed in triplicate, are presented in Table 10. As shown, surface expression of LDL receptor is significantly enhanced in the presence of peptide.

Table 10

Specific Anti-LDL Receptor Antibody Binding to the Cell Surface of Human Fibroblasts	
Peptide	Mean ± SEM (per 10,000 cells)
-	249 ± 243
+	1948 ± 213

To determine whether increased surface expression was due to an overall increase in the number of LDL receptors in the cell or an inhibition of internalization of the 20 receptor, the relative amounts of cell surface, internalized, and degraded LDL were examined as a function

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of peptide concentration. Normal human fibroblasts were seeded in 6-well tissue culture plates at 10,000 cells/well in 2.0 ml DMEM medium containing 10% FCS. After overnight incubation, the cells were washed in DMEM with 2% BSA and 5  $^{125}$ I-LDL added in the absence or presence of various concentrations of [Ala<sup>85</sup>]D<sup>k</sup>(69-85) peptide. Following incubation for 5 hr at 37°C, the relative amounts of cell surface and internalized LDL receptors in untreated cells were determined as the amount of dextran sulfate soluble and 10 dextran sulfate resistant  $^{125}$ I-LDL respectively. The amount of degraded LDL receptors was determined by detecting the amount of  $^{125}$ I-monotyrosine in the culture medium. All values obtained in samples containing peptide were set relative to samples prepared in the absence of peptide. As 15 shown in Figure 7, the amount of cell surface LDL significantly increased with an increase in peptide concentration up to a maximum surface expression (about 1.5 fold higher than controls) in the presence of about 30 mM peptide. Concurrently, the amount of internalized and 20 degraded LDL decreased with increasing peptide concentration. Thus, the increase in surface expression of LDL receptors in the presence of peptide is a result of the inhibition of internalization of the receptor.

Example 9. MHC Class I Peptide Regulation of the LDL  
25 "Scavenger" Receptor

The effect of [Ala<sup>85</sup>]D<sup>k</sup>(69-85) on internalization of the LDL scavenger receptor was examined in the murine macrophage cell line J774. Macrophages were incubated for 2 hr at 37°C in DMEM with 2% BSA with  $^{125}$ I-acetylated-LDL ( $\pm$  dextran 30 sulfate) in the absence or presence of peptide. The amount of specific binding of labeled LDL was determined by the ability of unlabeled LDL to compete away bound labeled LDL. The amounts of cell-associated and degraded LDL in the

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medium were determined as described above in Example 8. The results presented in Table 11 are the mean  $\pm$  SEM of three experiments performed with triplicate samples.

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Table 11

	Cell-Associated LDL				Degraded LDL		
	Pep-tide	La-beled	Labeled Remaining After Competition with Unlabeled	Spe-cific	La-beled	Labeled Remaining After Competition with Unlabeled	Spe-cific
-		100	27 ± 5	73	100	48 ± 6	52
5	+	97 ± 9	20 ± 3	77	38 ± 3	42 ± 6	-4

Thus, in the presence of peptide, the amount of LDL associated with the cell remains essentially the same; however, the level of LDL degradation is significantly decreased in the presence of peptide. These data imply 10 that, as for other receptors described above, [Ala<sup>85</sup>]D<sup>k</sup>(69-85) peptide increases the population of LDL receptors on the cell surface.

Example 10. Effect of MHC Class I Peptide on b<sub>2</sub>-Adrenergic Receptor (b<sub>2</sub>AR)

15 Chinese hamster ovary (CHO) cells expressing b<sub>2</sub>AR were incubated in DMEM with 5% FCS in the absence or presence of 30 mM of the [Ala<sup>85</sup>]-D<sup>k</sup>(69-85) peptide. The media was then supplemented with 5 mM isoproterenol to induce b<sub>2</sub>AR internalization. Incubation was continued for 0, 7, 20 and 20 60 min and then terminated by washing the cells with ice cold PBS. The cells were collected and subjected to a ligand binding assay utilizing <sup>125</sup>I-iodopindolol in the presence of 0.4 mM of a hydrophilic propanol derivative

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(e.g. CGP, a hydrophilic ligand used to label cell surface receptors). Ligand binding, as reflected by the distribution of receptors in the plasma membrane versus internalized receptors, was determined. The amount of 5 ligand binding was determined to be the same in both untreated control cells and cells exposed to peptide. Thus the surface expression and internalization of  $\beta_2$ AR is unaffected by the presence of peptide.

Example 11. Effect of Antibody to MHC Class I on Glucose

10 Uptake and Peptide Binding

The antibody Ox-18 [Sigma, St. Louis] has binding specificity for Class I MHC antigen of rats. The effect of this antibody on glucose uptake in rat adipose cells was examined. Rat adipose cells were prepared and seeded for 15 assays as described in Example 4. Briefly, rat adipose cells were obtained from epididymal fat pads and suspended in Krebs-Ringer HEPES buffer (KRH) with 5% bovine serum albumin at a lipocrit of 10% (final). The effect of the antibody was measured in cells maximally stimulated with 20 insulin (8 nM). After equilibration at 37°C for 30 min the cells were incubated for 30 min at 37°C with buffer (basal), 8 nM insulin plus varying concentrations of Ox-18 antibody. Samples containing 8 nM insulin plus either 30 mM [ $\text{Ala}^{85}$ ]-D<sup>k</sup>(69-85) peptide or 6 mg/ml Ir.Ab, an anti-mouse K<sup>k</sup> Class I 25 MHC antibody, were included as positive and negative controls, respectively. <sup>14</sup>C-D-glucose was added, the cells were incubated for an additional 30 min, harvested on oil and counted in a scintillation counter. All values were expressed relative to the basal level of glucose uptake.

30 In the presence of 30 mM [ $\text{Ala}^{85}$ ]-D<sup>k</sup>(69-85) peptide, glucose uptake levels were 60% over the basal levels while the anti-mouse MHC class I antibody had no affect on glucose uptake. However, the Ox-18 antibody significantly affected

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glucose uptake, with the increase of glucose uptake relative to basal levels increasing with increasing concentration of antibody to a maximal stimulation of glucose uptake at about 60% above basal levels. These data support the theory that 5 this anti-MHC Class I antibody and the [Ala<sup>85</sup>]-D<sup>k</sup>(69-85) stimulate glucose uptake by similar mechanisms.

In similar assays the effect of incubation with [Ala<sup>85</sup>]-D<sup>k</sup>(69-85) peptide and the Ox-18 antibody either alone or in combination on the glucose uptake level of rat adipocytes 10 was examined. The protocol described fo the antibody experiments above was again employed. However, in these experiments, the following samples were prepared: no peptide (basal level control), 30 mM activated [Ala<sup>85</sup>]-D<sup>k</sup>(69-85) peptide plus 8 nM insulin, 2 mg/ml Ox-18 antibody plus 15 8 nM insulin, 30 mM activated [Ala<sup>85</sup>]-D<sup>k</sup>(69-85) peptide plus 2 mg/ml Ox-18 antibody plus 8 nM insulin, and 2 mg/ml Ir.Ab (anti-mouse K<sup>k</sup> Class I MHC antibody) plus 8 nM insulin. The results, presented in Table 12, are expressed as a percent of the basal level of glucose uptake.

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Table 12.

Effect of Antibody to MHC Class I (Ox-18) on Glucose Uptake and $^{125}\text{I}$ -[Ala <sup>85</sup> ]-D <sup>k</sup> (69-85) Peptide Binding in Rat Adipose Cells					
	Basal Level	Peptide (30 mM)	Ox-18 (2 mg/ml)	Peptide + Ox-18	Ir. Ab (2 mg/ml)
Glucose Uptake	100	139±10	142±7	161±7	102±4
Specific Binding of Peptide	100	0	19±4	0	93±8

As seen above, [Ala<sup>85</sup>]-D<sup>k</sup>(69-85) peptide and Ox-18 enhanced glucose uptake levels to similar levels. However, in samples containing both peptide and Ox-18 antibody, glucose uptake levels were only slightly enhanced relative to peptide alone or antibody alone. Thus, these data imply that peptide and Ox-18 antibody act by similar mechanisms and are competing for sites of action.

To examine the possibility that the [Ala<sup>85</sup>]-D<sup>k</sup>(69-85) peptide and Ox-18 antibody share the same or similar binding sites, a competitive binding assay with rat adipose cells was performed. The samples described above were again prepared. Rather than adding  $^{14}\text{C}$ -D-glucose,  $^{125}\text{I}$ -[Ala<sup>85</sup>]-D<sup>k</sup>(69-85) (approximately 25,000 cpm/sample) was added to a

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concentration of 30 mM to all samples. Incubation at 37 °C continued for 10 min. The cells were diluted twofold with cold KRHB (5% BSA) at pH 7.4 or pH 1.8 and held once for 10 min. Finally, the cells were separated from the supernatant 5 by centrifugation through oil and the number of specific counts determined using a gamma scintillation counter. All values were set relative to specific binding of peptide in the basal level samples (100%). As seen from the data in Table 12, the presence of the control antibody, Ir.Ab had no 10 significant effect on the binding of labeled peptide. In contrast, the Ox-18 antibody reduced the specific binding of labeled peptide to only about 19% of control levels. While binding of the [Ala<sup>85</sup>]-D<sup>k</sup>(69-85) peptide was not completely inhibited by the Ox-18 antibody, this significant reduction 15 of binding suggests that the antibody and the peptide competitively bind for similar sites.

It is evident from the above results that surface membrane receptors, particularly those receptors inactivated by internalization and involving transduction of signals as 20 exemplified by the insulin receptor, EGF receptor, IGF-I receptor, IGF-II receptor, LDL receptor and LDL scavenger receptor are modulated by MHC Class I antigen and peptides derived therefrom, particularly H-2D and -L of mice and HLA-B and -C of humans. A wide variety of physiological 25 processes, both in vitro and in vivo, may be regulated by controlling the interaction between the appropriate Class I antigen and the surface membrane receptor, by a variety of techniques which allow for the enhancement or reduction of the interaction between the Class I antigen and the surface 30 membrane receptor.

All publications and patent applications mentioned in this specification are indicative of the level of skill of those skilled in the art to which this invention pertains. All publications and patent applications are herein

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incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

5 Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims.

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WHAT IS CLAIMED IS:

1. A method for regulating a surface receptor response of a mammalian cell, said method comprising:

5 adding to said mammalian cells an agent capable of modulating the interaction between (1) a human major histocompatibility complex Class I antigen or other mammalian equivalent thereof and (2) said surface receptor on said cell by varying the number of said antigens on the surface of said cell or by adding an agent capable of 10 inhibiting said interaction, whereby said receptor response is modulated.

2. A method according to Claim 1, wherein said agent affects the number of said antigens on said cell.

3. A method according to Claim 1, wherein said agent is 15 an antibody having binding specificity to one of said antigen or surface receptor and inhibiting said interaction.

4. A method according to Claim 1, wherein said agent is an oligopeptide binding to said antigen and inhibiting said interaction.

20 5. A method according to Claim 1, wherein said receptor is internalized.

6. A method for regulating an endocrine, paracrine or autocrine surface receptor response of a human cell, said method comprising:

25 adding to said mammalian cells an agent capable of modulating the interaction between (1) a human major histocompatibility complex Class I antigen or other mammalian equivalent thereof and (2) said surface receptor on said cell by varying the number of said antigens on the 30 surface of said cell or by adding an agent capable of

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inhibiting said interaction, whereby said receptor response is modulated.

7. A method according to Claim 6, wherein said agent is an oligopeptide of at least 6 amino acids, said oligopeptide having substantially the same amino acid sequence as an  $\alpha_1$  domain of a human major histocompatibility complex Class I antigen or other mammalian equivalent thereof.

8. A method according to Claim 7, wherein said amino acid sequence of said oligopeptide is within the sequence of the amino acid sequence from amino acid 55 to 90 of said  $\alpha_1$  domain.

9. A method according to Claim 7, wherein said amino acid sequence of said oligopeptide is substantially the same as an amino acid sequence of said  $\alpha_1$  domain and is of at least about 8 to 24 amino acids and includes the sequence R Y X, wherein X may be Y, A or an amino group.

10. A method according to Claim 7, wherein said amino acid sequence of said oligopeptide is substantially the same as an amino acid sequence of said  $\alpha_1$  domain and is of at least about 8 to 24 amino acids and includes the sequence R Y A.

11. A method for regulating an insulin receptor response of a mammalian cell, said method comprising:

adding to said mammalian cells an agent capable of modulating the interaction between (1) a human major histocompatibility complex Class I antigen or other mammalian equivalent thereof and (2) said insulin receptor on said cell by varying the number of said antigens on the surface of said cell or by adding an agent capable of inhibiting said interaction, whereby said insulin receptor response is modulated.

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12. A method according to Claim 11, wherein said agent is an oligopeptide of at least about 8 amino acids having an amino acid sequence substantially the same amino acid sequence of the  $\alpha_1$  domain of said antigen.

5 13. A method according to Claim 12, wherein the amino acid sequence of said oligopeptide is within the sequence of the amino acid sequence of said  $\alpha_1$  domain of said antigen from amino acid 55 to 90 and includes the sequence R Y X, wherein X may be Y, A or an amino group.

10 14. A method according to Claim 12, wherein the amino acid sequence of said oligopeptide is within the sequence of the amino acid sequence of said  $\alpha_1$  domain of said antigen from amino acid 55 to 90 and includes the sequence R Y A.

15 15. A method according to Claim 12, wherein the amino acid sequence of said oligopeptide is substantially the same as the sequence from amino acid 61 to 85 of the  $\alpha^1$  domain of said antigen.

20 16. A method according to Claim 12, wherein the amino acid sequence of said oligopeptide is substantially the same as the sequence from amino acid 62 to 85 of the  $\alpha^1$  domain of said antigen.

25 17. An oligopeptide of at least 8 amino acids comprising a sequence of the  $\alpha_1$  domain of a human major histocompatibility complex Class I antigen or mammalian equivalent thereof consisting essentially of a sequence contained within one of the following sequence units:

(a) W aa<sup>61</sup> aa<sup>62</sup> aa<sup>63</sup> aa<sup>64</sup> aa<sup>65</sup> aa<sup>66</sup> aa<sup>67</sup> aa<sup>68</sup> aa<sup>69</sup> aa<sup>70</sup> aa<sup>71</sup> aa<sup>72</sup> aa<sup>73</sup> aa<sup>74</sup>

aa<sup>75</sup> aa<sup>76</sup> aa<sup>77</sup> aa<sup>78</sup> aa<sup>79</sup> aa<sup>80</sup> aa<sup>81</sup> aa<sup>82</sup> aa<sup>83</sup> aa<sup>84</sup> aa<sup>85</sup>

30 (b) E Q aa<sup>73</sup> F R V aa<sup>77</sup> aa<sup>78</sup> R aa<sup>80</sup> aa<sup>81</sup> aa<sup>82</sup> R Y aa<sup>85</sup>

wherein:

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aa<sup>61</sup> is D or E;  
aa<sup>62</sup> is K, R, G or A;  
aa<sup>63</sup> is any aliphatic amino acid other than basic of  
from 4 to 6 carbon atoms;  
5 aa<sup>64</sup> is S, T or M;  
aa<sup>65</sup> is any polar or basic amino acid of from 4 to 6  
carbon atoms;  
aa<sup>66</sup> is any aliphatic amino acid of from 4 to 6 carbon  
atoms;  
10 aa<sup>67</sup> is any neutral aliphatic or aromatic amino acid;  
aa<sup>68</sup> is K or R;  
aa<sup>69</sup> is any aliphatic neutral amino acid other than A;  
aa<sup>70</sup> is any aliphatic amino acid other than acidic of  
from 3 to 6 carbon atoms;  
15 aa<sup>71</sup> is any aliphatic amino acid other than basic;  
aa<sup>72</sup> is N or Q;  
aa<sup>73</sup> is S, T, F, Y, H or W;  
aa<sup>74</sup> is F, Y or W, particularly F;  
aa<sup>75</sup> is K or R;  
20 aa<sup>76</sup> is an aliphatic amino acid other than basic of from  
4 to 6 carbon atoms;  
aa<sup>77</sup> is a polar aliphatic amino acid of from 3 to 6  
carbon atoms;  
aa<sup>78</sup> is a neutral aliphatic amino acid of from 5 to 6  
25 carbon atoms;  
aa<sup>79</sup> is K or R;  
aa<sup>80</sup> is a neutral aliphatic amino acid of from 3 to 6  
carbon atoms other than A;  
aa<sup>81</sup> is a neutral aliphatic non-polar amino acid other  
30 than A;  
aa<sup>82</sup> is an aliphatic amino acid other than acidic and  
other than A;  
aa<sup>83</sup> is an aliphatic amino acid other than acidic and  
other than A;  
35 aa<sup>84</sup> is an aromatic amino acids; and  
aa<sup>85</sup> is an aromatic amino acid or A;

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with the proviso that there are not more than three amino acid mutations as deletions, insertions or substitutions.

18. An oligopeptide according to Claim 17, wherein said 5 oligopeptide sequence comes within the following unit:

W D/E R aa<sup>43</sup> T Q/R aa<sup>46</sup> aa<sup>47</sup> K aa<sup>49</sup> aa<sup>50</sup> aa<sup>51</sup> Q S/T F R V/E aa<sup>57</sup> L R T L L/R G/R Y Y/A.

where aa/aa intends either amino acid may be present.

19. A peptide conjugate comprising an oligopeptide 10 according to Claim 18 covalently bonded to at least one amino acid, wherein said amino acid is other than the wild type amino acid.

20. A peptide conjugate according to Claim 19, wherein 15 said at least one amino acid is an immunogenic polypeptide capable of stimulating an immune response in a vertebrate host.

21. A method for regulating an epidermal growth factor receptor response of a mammalian cell, said method comprising:

20 adding to said mammalian cells an agent capable of modulating the interaction between (1) a human major histocompatibility complex Class I antigen or other mammalian equivalent thereof and (2) said epidermal growth factor receptor on the surface of said cell by varying the 25 number of said antigens on the surface or by adding an agent capable of inhibiting said interaction, whereby said receptor response is modulated.

22. A method according to claim 21, wherein said agent is an oligopeptide of at least 6 amino acids, said 30 oligopeptide having substantially the same amino acid sequence as an  $\alpha_1$  domain of a human major histocompatibility

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complex Class I antigen or other mammalian equivalent thereof.

23. A method according to Claim 22, wherein said amino acid sequence of said oligopeptide is within the sequence of 5 the amino acid sequence from amino acid 55 to 90 of said  $\alpha_1$  domain .

24. A method according to Claim 23, wherein said amino acid sequence of said oligopeptide is substantially the same as an amino acid sequence of said  $\alpha_1$  domain and is of at 10 least about 8 to 24 amino acids and includes the sequence R Y X, wherein X may be Y, A or an amino group.

25. A method according to Claim 23, wherein said amino acid sequence of said oligopeptide is substantially the same as an amino acid sequence of said  $\alpha_1$  domain and is of at 15 least about 8 to 24 amino acids and includes the sequence R Y A.

26. A method for regulating an insulin-like growth factor I receptor response of a mammalian cell, said method comprising:

20 adding to said mammalian cells an agent capable of modulating the interaction between (1) a human major histocompatibility complex Class I antigen or other mammalian equivalent thereof and (2) said surface receptor on said cell by varying the number of said antigens on the 25 surface of said cell or by adding an agent capable of inhibiting said interaction, whereby said receptor response is modulated.

27. A method according to claim 26, wherein said agent is an oligopeptide of at least 6 amino acids, said 30 oligopeptide having substantially the same amino acid sequence as an  $\alpha_1$  domain of a human major histocompatibility

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complex Class I antigen or other mammalian equivalent thereof.

28. A method according to Claim 27, wherein said amino acid sequence of said oligopeptide is within the sequence of 5 the amino acid sequence from amino acid 55 to 90 of said  $\alpha_1$  domain .

29. A method according to Claim 27, wherein said amino acid sequence of said oligopeptide is substantially the same as an amino acid sequence of said  $\alpha_1$  domain and is of at 10 least about 8 to 24 amino acids and includes the sequence R Y X, wherein X may be Y, A or an amino group.

30. A method according to Claim 27, wherein said amino acid sequence of said oligopeptide is substantially the same as an amino acid sequence of said  $\alpha_1$  domain and is of 15 at least about 8 to 24 amino acids and includes the sequence R Y A.

31. A method for regulating surface expression of a glucose transporter of a mammalian cell, said method comprising:

20 adding to said mammalian cells an agent capable of modulating the interaction between (1) a human major histocompatibility complex Class I antigen or other mammalian equivalent thereof and (2) said glucose transporter on said cell by varying the number of said 25 antigens on the surface of said cell or by adding an agent capable of inhibiting said interaction, whereby said receptor response is modulated.

32. A method according to claim 31, wherein said agent is an oligopeptide of at least 6 amino acids, said 30 oligopeptide having substantially the same amino acid sequence as an  $\alpha_1$  domain of a human major histocompatibility

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complex Class I antigen or other mammalian equivalent thereof.

33. A method according to Claim 32, wherein said amino acid sequence of said oligopeptide is within the sequence of 5 the amino acid sequence from amino acid 55 to 90 of said  $\alpha_1$  domain.

34. A method according to Claim 32, wherein said amino acid sequence of said oligopeptide is substantially the same as an amino acid sequence of said  $\alpha_1$  domain and is of at 10 least about 8 to 24 amino acids and includes the sequence R Y X, wherein X may be Y, A or an amino group.

35. A method according to Claim 32, wherein said amino acid sequence of said oligopeptide is substantially the same as an amino acid sequence of said  $\alpha_1$  domain and is of 15 at least about 8 to 24 amino acids and includes the sequence R Y A.

36. A method for regulating an insulin-like growth factor II receptor response of a mammalian cell, said method comprising:

20 adding to said mammalian cells an agent capable of modulating the interaction between (1) a human major histocompatibility complex Class I antigen or other mammalian equivalent thereof and (2) said surface receptor on said cell by varying the number of said antigens on the 25 surface of said cell or by adding an agent capable of inhibiting said interaction, whereby said receptor response is modulated.

37. A method according to claim 36, wherein said agent is an oligopeptide of at least 6 amino acids, said 30 oligopeptide having substantially the same amino acid sequence as an  $\alpha_1$  domain of a human major histocompatibility

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complex Class I antigen or other mammalian equivalent thereof.

38. A method according to Claim 37, wherein said amino acid sequence of said oligopeptide is within the sequence of 5 the amino acid sequence from amino acid 55 to 90 of said  $\alpha_1$  domain.

39. A method according to Claim 37, wherein said amino acid sequence of said oligopeptide is substantially the same as an amino acid sequence of said  $\alpha_1$  domain and is of at 10 least about 8 to 24 amino acids and includes the sequence R Y X, wherein X may be Y, A or an amino group.

40. A method according to Claim 37, wherein said amino acid sequence of said oligopeptide is substantially the same as an amino acid sequence of said  $\alpha_1$  domain and is of 15 at least about 8 to 24 amino acids and includes the sequence R Y A.

41. A method for regulating a low-density lipoprotein receptor response of a mammalian cell, said method comprising:

20 adding to said mammalian cells an agent capable of modulating the interaction between (1) a human major histocompatibility complex Class I antigen or other mammalian equivalent thereof and (2) said surface receptor on said cell by varying the number of said antigens on the 25 surface of said cell or by adding an agent capable of inhibiting said interaction, whereby said receptor response is modulated.

42. A method according to claim 41, wherein said agent is an oligopeptide of at least 6 amino acids, said 30 oligopeptide having substantially the same amino acid sequence as an  $\alpha_1$  domain of a human major histocompatibility

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complex Class I antigen or other mammalian equivalent thereof.

43. A method according to Claim 42, wherein said amino acid sequence of said oligopeptide is within the sequence of 5 the amino acid sequence from amino acid 55 to 90 of said  $\alpha_1$  domain.

44. A method according to Claim 42, wherein said amino acid sequence of said oligopeptide is substantially the same as an amino acid sequence of said  $\alpha_1$  domain and is of at 10 least about 8 to 24 amino acids and includes the sequence R Y X, wherein X may be Y, A or an amino group.

45. A method according to Claim 42, wherein said amino acid sequence of said oligopeptide is substantially the same as an amino acid sequence of said  $\alpha_1$  domain and is of 15 at least about 8 to 24 amino acids and includes the sequence R Y A.

46. A method for regulating a low-density lipoprotein scavenger receptor response of a mammalian cell, said method comprising:

20 adding to said mammalian cells an agent capable of modulating the interaction between (1) a human major histocompatibility complex Class I antigen or other mammalian equivalent thereof and (2) said surface receptor on said cell by varying the number of said antigens on the 25 surface of said cell or by adding an agent capable of inhibiting said interaction, whereby said receptor response is modulated.

47. A method according to claim 46, wherein said agent is an oligopeptide of at least 6 amino acids, said 30 oligopeptide having substantially the same amino acid sequence as an  $\alpha_1$  domain of a human major histocompatibility

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complex Class I antigen or other mammalian equivalent thereof.

48. A method according to Claim 47, wherein said amino acid sequence of said oligopeptide is within the sequence of 5 the amino acid sequence from amino acid 55 to 90 of said  $\alpha_1$  domain.

49. A method according to Claim 47, wherein said amino acid sequence of said oligopeptide is substantially the same as an amino acid sequence of said  $\alpha_1$  domain and is of at 10 least about 8 to 24 amino acids and includes the sequence R Y X, wherein X may be Y, A or an amino group.

50. A method for inhibiting surface receptor-mediated entry of a virus into a mammalian cell, said method 15 comprising:

adding to said mammalian cells an agent capable of modulating the interaction between (1) a human major histocompatibility complex Class I antigen or other mammalian equivalent thereof and (2) said surface receptor 20 on said cell by varying the number of said antigens on the surface of said cell or by adding an agent capable of inhibiting said interaction, whereby viral entry is inhibited.

51. A method according to claim 50, wherein said agent 25 is an oligopeptide of at least 6 amino acids, said oligopeptide having substantially the same amino acid sequence as an  $\alpha_1$  domain of a human major histocompatibility complex Class I antigen or other mammalian equivalent thereof.

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52. A method according to Claim 51, wherein said amino acid sequence of said oligopeptide is within the sequence of the amino acid sequence from amino acid 55 to 90 of said a<sub>1</sub> domain .

5 53. A method according to Claim 51, wherein said amino acid sequence of said oligopeptide is substantially the same as an amino acid sequence of said a<sub>1</sub> domain and is of at least about 8 to 24 amino acids and includes the sequence R Y X, wherein X may be Y, A or an amino group.

10 54. A method according to Claim 51, wherein said amino acid sequence of said oligopeptide is substantially the same as an amino acid sequence of said a<sub>1</sub> domain and is of at least about 8 to 24 amino acids and includes the sequence R Y A.

15 55. A method for treatment of a patient having a defect in function of a receptor expressed on a cell surface, said method comprising the step of:

20 administering to said patient a drug in an amount effective to modulate the interaction between (1) a human major histocompatibility complex Class I antigen or other mammalian equivalent thereof and (2) said surface receptor, wherein said modulating compensates for said defect in receptor function.

25 56. A method according to Claim 55, wherein said drug is an oligopeptide.

57. A method according to Claim 56, wherein said oligopeptide is of at least about 6 amino acids having an amino acid sequence substantially the same as an amino acid sequence of the a' domain of said antigen.

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58. A method according to Claim 55, wherein said patient is a Type 2 diabetes patient.

59. A method according to Claim 58, wherein said receptor is insulin receptor.

60. A method according to Claim 58, wherein said receptor is a glucose transporter.

61. A method according to Claim 55, wherein said patient is a Type I diabetes patient and said receptor is insulin receptor and said method further comprises the step of administering an effective amount of insulin to said patient.

62. A method according to Claim 55, wherein said receptor is a receptor for a drug.

15 63. A method for manipulating a physiological process in a patient, said manipulating being accomplished by modulating cell surface expression of receptors mediating said physiological process, said method comprising the step of:

20 administering to said patient a drug in an amount effective to modulate cell surface expression of said receptors, said drug being capable of modulating the interaction between (1) a human major histocompatibility complex Class I antigen or other mammalian equivalent 25 thereof and (2) said surface receptor, wherein said modulating of said cell surface expression of said receptors achieves said manipulation of said physiological process.

64. A method according to Claim 63, wherein said physiological process is wound healing.

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65. A method according to Claim 64, wherein said receptor is epidermal growth factor receptor.

66. A method according to Claim 64, wherein said receptor is transforming growth factor a receptor.

5 67. A method according to Claim 64, wherein said receptor is platelet-derived growth factor receptor.

68. A method according to Claim 63, wherein said receptor is transferrin receptor.

10 69. A method according to Claim 63, wherein said receptor is low-density lipoprotein scavenger receptor.

70. A method for screening for drugs capable of inhibiting interaction between a major histocompatibility Class I antigen and a cell surface receptor, thereby modulating cell surface expression of said receptor, said  
15 method comprising the steps of:

binding to an insoluble support a peptide having activity in modulation of cell surface expression of said receptor;

20 adding to said bound peptide, a drug candidate and a labeled agent in amounts varying relative to one another, said agent having binding specificity to said bound peptide; and

detecting an amount of labeled agent bound in the presence and in the absence of said drug candidate;

25 wherein if said detected amount of labeled agent is less in the presence of said drug candidate relative to the amount of labeled agent detected in the absence of said drug candidate, said drug candidate is capable mediating modulation of said cell surface expression of  
30 said receptor.

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71. A method according to Claim 70, wherein said agent is an oligopeptide.

72. A method according to Claim 70, wherein said agent is an antibody.

5        73. A method for screening for drugs capable of inhibiting interaction between a major histocompatibility Class I antigen and a cell surface receptor, thereby modulating cell surface expression of said receptor, said method comprising the steps of:

10        binding to an insoluble support substantially purified MHC Class I antigen having activity in modulation of cell surface expression of said receptor;

              adding to said bound antigen, a drug candidate and a labeled agent in amounts varying relative to one

15        another, said agent having binding specificity to said bound peptide; and

              detecting an amount of labeled agent bound in the presence and in the absence of said drug candidate;

              wherein if said detected amount of labeled agent 20 is less in the presence of said drug candidate relative to the amount of labeled agent detected in the absence of said drug candidate, said drug candidate is capable mediating modulation of said cell surface expression of said receptor.

25        74. A method according to Claim 73, wherein said agent is an oligopeptide.

75. A method according to Claim 73, wherein said agent is an antibody.

30        76. A method for screening for drugs capable of inhibiting interaction between a major histocompatibility Class I antigen and a cell surface receptor, thereby

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modulating cell surface expression of said receptor, said method comprising the steps of:

binding to an insoluble support cells, said receptor being present on the surface of said cells;

5 adding to said bound peptide, a drug candidate and a labeled agent in amounts varying relative to one another, said agent having binding specificity to said bound peptide; and

detecting an amount of labeled agent bound in the  
10 presence and in the absence of said drug candidate;

wherein if said detected amount of labeled agent is less in the presence of said drug candidate relative to the amount of labeled agent detected in the absence of said drug candidate, said drug candidate is capable

15 mediating modulation of said cell surface expression of said receptor.

77. A method according to Claim 76, wherein said agent is an oligopeptide.

78. A method according to Claim 76, wherein said agent  
20 is an antibody.

79. A method for screening for drugs capable of inhibiting interaction between a major histocompatibility Class I antigen and a cell surface receptor, thereby modulating cell surface expression of said receptor, said  
25 method comprising the steps of:

adding varying amounts of said drug candidate to a solution of first peptide, said first peptide having activity in modulation of cell surface expression of said receptor;

30 adding varying amounts of a second peptide, said second peptide lacking the ability to bind to said first peptide;

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wherein if aggregation of said first peptide is inhibited in the presence of said drug candidate, and said amount of the drug candidate added to achieve said inhibition is less than said amount of said second peptide 5 which results in non-specific inhibition of aggregation of said first peptide, said drug candidate is capable of mediating modulation of said cell surface expression of said receptor.

80. A method of administration of a self-aggregating 10 peptide to a mammalian host, said method comprising the steps of:

producing a gel comprising said self-aggregating peptide, said oligopeptide being of at least 6 amino acids and having substantially the same amino acid sequence as 15 an  $\alpha_1$  domain of a human major histocompatibility complex Class I antigen or other mammalian equivalent thereof; and administering said gel to said mammalian host.

81. A method according to Claim 80, wherein said peptide is capable of modulation of surface expression of 20 a receptor.

82. A method according to Claim 80, wherein said gel further comprises a drug.

83. A method according to Claim 82, wherein said peptide is capable of modulation of surface expression of 25 a receptor and said drug is selected so as to act in synergy with said active peptide.

84. A method according to Claim 80, wherein said administering is by subcutaneous injection.

85. A method according to Claim 80, wherein said 30 administering is by topical application.

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86. A method according to Claim 85, wherein said administering is by means of transdermal transport.

SUBSTITUTE SHEET (RULE 26)

**AMENDED CLAIMS**

[ received by the International Bureau on 30 January 1995(30.01.95);  
original claims 1-86 replaced by amended claims 1-9 (4 pages)]

1. A composition for use in a method for regulating a surface receptor response of a mammalian cell, said method comprising:
  - adding to said mammalian cells an agent capable of modulating the interaction
  - between (1) a human major histocompatibility complex Class I antigen or other mammalian equivalent thereof and (2) said surface receptor on said cell, wherein said surface receptor is one of insulin-like growth factor I2, a glucose transporter of a mammalian cell, an insulin-like growth factor II receptor, a low-density lipoprotein receptor, a low-density lipoprotein scavenger receptor and a surface receptor mediating entry of a virus, by varying the number of said antigens on the surface of said cell or by adding an agent capable of inhibiting said interaction, wherein said agent is characterized as competitive for said interaction with an oligopeptide of at least 6 amino acids, said oligopeptide having substantially the amino acid sequence of an  $\alpha_1$  domain of a human major histocompatibility complex Class I antigen or other mammalian equivalent thereof;
  - whereby said receptor response is modulated.
2. A composition according to Claim 1, wherein said amino acid sequence of said oligopeptide is substantially the same as an amino acid sequence of said  $\alpha_1$  domain and is of at least about 8 to 24 amino acids and includes the sequence R Y X, wherein X may be any amino acid.
3. A composition according to Claim 2, wherein said amino acid sequence of said oligopeptide includes the sequence R Y A.
4. A composition for use in a method for manipulating wound healing in a patient, said manipulating being accomplished by modulating cell surface expression of receptors mediating said wound healing, said method comprising the step of:
  - administering to said patient a drug in an amount effective to modulate cell surface expression of said receptors, said drug being capable of modulating the interaction between (1) a human major histocompatibility complex Class I antigen or other mammalian equivalent thereof and (2) said surface receptor, wherein said receptor is selected from the group consisting of epidermal growth factor receptor, transforming growth factor  $\alpha$  receptor, platelet-derived growth factor receptor, transferrin receptor and low-density lipoprotein scavenger receptor, and wherein said drug is characterized as competitive for said interaction with an oligopeptide of at least 6 amino acids, said oligopeptide having substantially the amino acid sequence of an  $\alpha_1$  domain of a human major histocompatibility complex Class I antigen or other mammalian equivalent thereof;

wherein said modulating of said cell surface expression of said receptors achieves said manipulation of said wound healing.

5. A method for screening for drugs capable of inhibiting interaction  
between a major histocompatibility Class I antigen and a cell surface receptor, thereby  
modulating cell surface expression of said receptor, said method comprising the steps  
of:

binding to an insoluble support one of (a) a peptide having activity in  
modulation of cell surface expression of said receptor, (b) substantially purified MHC  
10 Class I antigen having activity in modulation of cell surface expression of said receptor,  
or (c) cells, said receptor being present on the surface of said cells;  
adding to said bound (a), (b) or (c), a drug candidate and a labeled agent in  
amounts varying relative to one another, said agent having binding specificity to said  
bound (a), (b) or (c); and  
15 detecting an amount of labeled agent bound in the presence and in the absence  
of said drug candidate;  
wherein if said detected amount of labeled agent is less in the presence of said  
drug candidate relative to the amount of labeled agent detected in the absence of said  
drug candidate, said drug candidate is capable mediating modulation of said cell surface  
20 expression of said receptor.

6. A composition for use in a method of administration of a self-aggregating peptide to a mammalian host, said method comprising the steps of:

producing a gel comprising said self-aggregating peptide, said oligopeptide  
25 being of at least 6 amino acids and having substantially the same amino acid sequence  
as an  $\alpha_1$  domain of a human major histocompatibility complex Class I antigen or other  
mammalian equivalent thereof, wherein said peptide is capable of modulation of surface  
expression of a receptor, wherein said receptor is one of insulin-like growth factor I2, a  
glucose transporter of a mammalian cell, an insulin-like growth factor II receptor, a  
30 low-density lipoprotein receptor, a low-density lipoprotein scavenger receptor and a  
surface receptor mediating entry of a virus; and  
administering said gel to said mammalian host.

7. A composition according to Claim 6, wherein said gel further  
35 comprises a drug selected so as to act in synergy with said active peptide.

8. A composition according to Claim 7, wherein said administering is by  
one of subcutaneous injection, topical application and transdermal transport.

9. An oligopeptide capable of modulating the interaction between (1) a human major histocompatibility complex Class I antigen or other mammalian equivalent thereof and (2) said surface receptor on said cell, wherein said surface receptor is one of insulin-like growth factor I2, a glucose transporter of a mammalian  
5 cell, an insulin-like growth factor II receptor, a low-density lipoprotein receptor, a low-density lipoprotein scavenger receptor and a surface receptor mediating entry of a virus, comprising:

an oligopeptide of at least 8 amino acids comprising a sequence of the  $\alpha_1$  domain of a human major histocompatibility complex Class I antigen or mammalian  
10 equivalent thereof consisting essentially of a sequence contained within one of the following sequence units:

(a) W aa<sup>61</sup> aa<sup>62</sup> aa<sup>63</sup> aa<sup>64</sup> aa<sup>65</sup> aa<sup>66</sup> aa<sup>67</sup> aa<sup>68</sup> aa<sup>69</sup> aa<sup>70</sup> aa<sup>71</sup> aa<sup>72</sup> aa<sup>73</sup> aa<sup>74</sup>  
aa<sup>75</sup> aa<sup>76</sup> aa<sup>77</sup> aa<sup>78</sup> aa<sup>79</sup> aa<sup>80</sup> aa<sup>81</sup> aa<sup>82</sup> aa<sup>83</sup> aa<sup>84</sup> aa<sup>85</sup>

(b) E Q aa<sup>73</sup> F R V aa<sup>77</sup> aa<sup>78</sup> R aa<sup>80</sup> aa<sup>81</sup> aa<sup>82</sup> R Y aa<sup>85</sup>

15 wherein:

aa<sup>61</sup> is D or E;

aa<sup>62</sup> is K, R, G or A;

aa<sup>63</sup> is any aliphatic amino acid other than basic of from 4 to 6 carbon atoms;

aa<sup>64</sup> is S, T or M;

20 aa<sup>65</sup> is any polar or basic amino acid of from 4 to 6 carbon atoms;

aa<sup>66</sup> is any aliphatic amino acid of from 4 to 6 carbon atoms;

aa<sup>67</sup> is any neutral aliphatic or aromatic amino acid;

aa<sup>68</sup> is K or R;

aa<sup>69</sup> is any aliphatic neutral amino acid other than A;

25 aa<sup>70</sup> is any aliphatic amino acid other than acidic of from 3 to 6 carbon atoms;

aa<sup>71</sup> is any aliphatic amino acid other than basic;

aa<sup>72</sup> is N or Q;

aa<sup>73</sup> is S, T, F, Y, H or W;

aa<sup>74</sup> is F, Y or W, particularly F;

30 aa<sup>75</sup> is K or R;

aa<sup>76</sup> is an aliphatic amino acid other than basic of from 4 to 6 carbon atoms;

aa<sup>77</sup> is a polar aliphatic amino acid of from 3 to 6 carbon atoms;

aa<sup>78</sup> is a neutral aliphatic amino acid of from 5 to 6 carbon atoms;

aa<sup>79</sup> is K or R;

35 aa<sup>80</sup> is a neutral aliphatic amino acid of from 3 to 6 carbon atoms other than A;

aa<sup>81</sup> is a neutral aliphatic non-polar amino acid other than A;

aa<sup>82</sup> is an aliphatic amino acid other than acidic and other than A;

aa<sup>83</sup> is an aliphatic amino acid other than acidic and other than A;

aa<sup>84</sup> is an aromatic amino acids; and

aa<sup>85</sup> is any amino acid excluding Y;  
with the proviso that there are not more than three amino acid mutations as  
deletions, insertions or substitutions.

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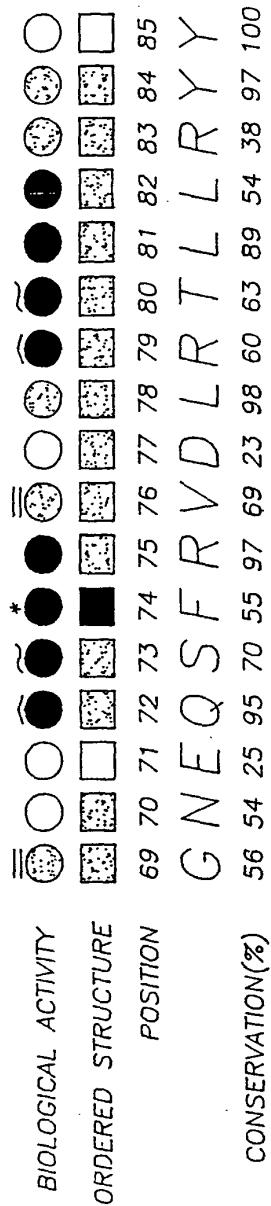


FIG. - 1A

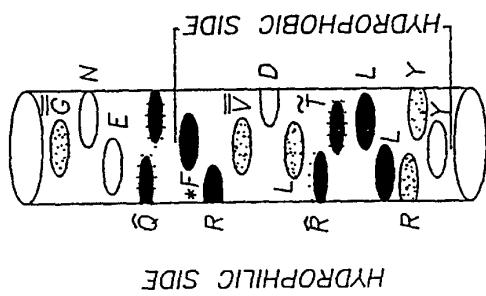


FIG. - 1 B

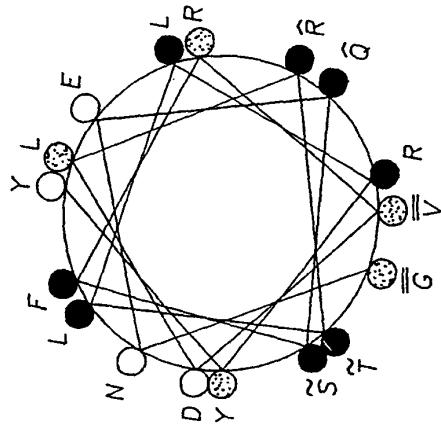


FIG. - 1 C

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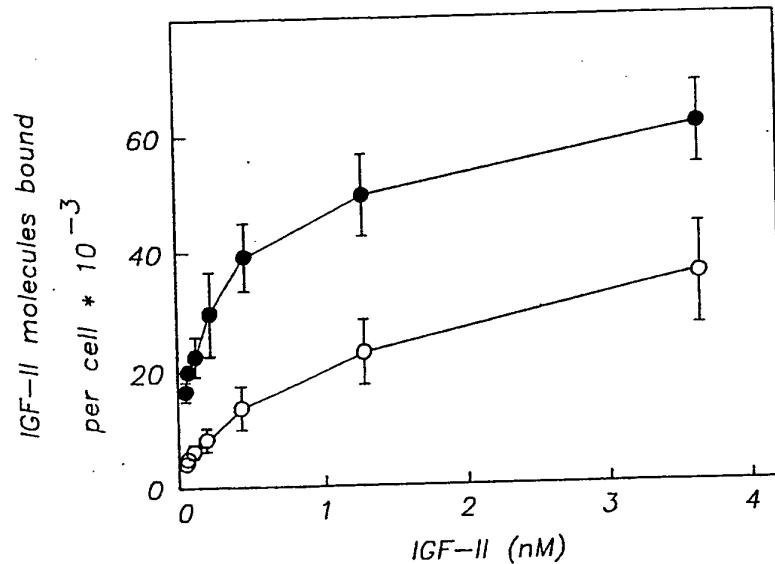


FIG.-2A

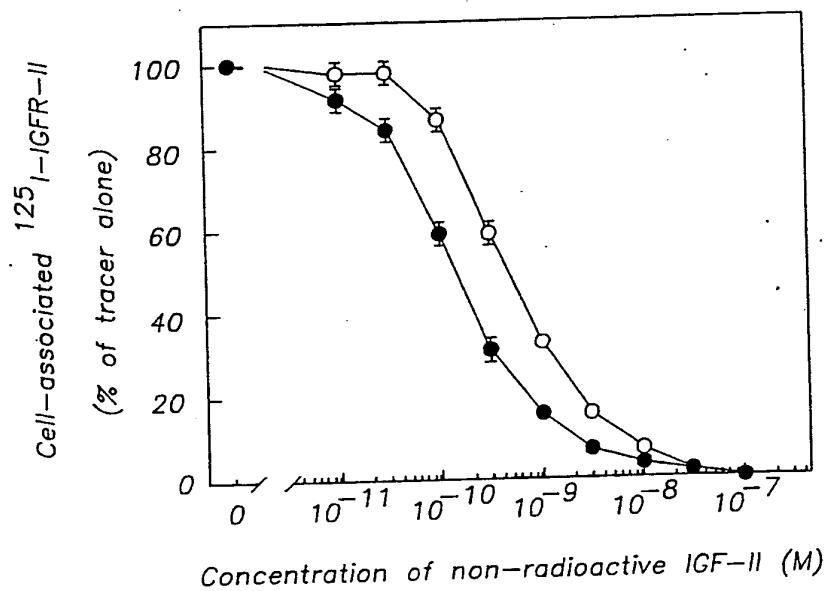


FIG.-2B

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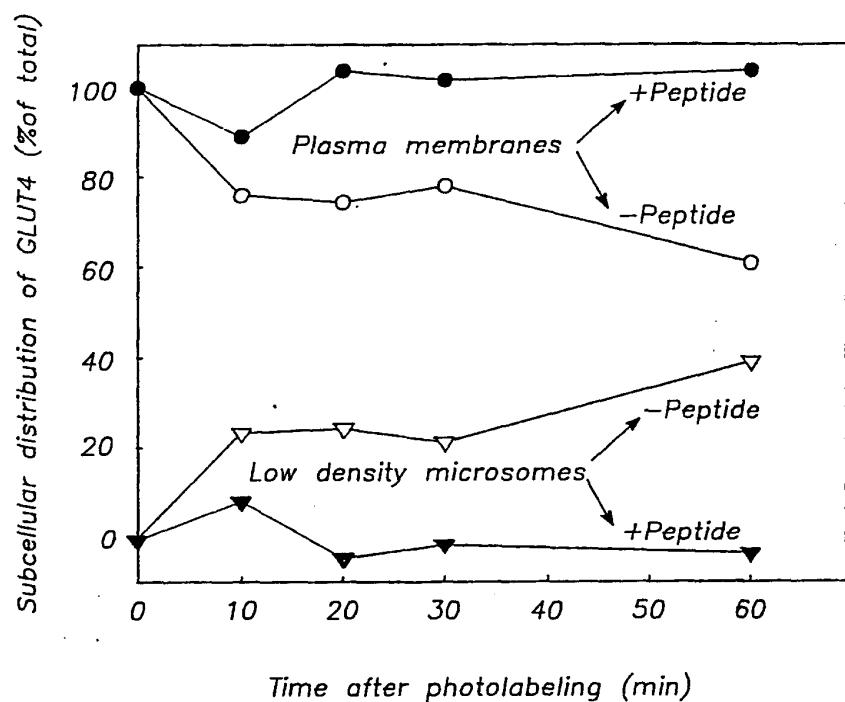


FIG. -3

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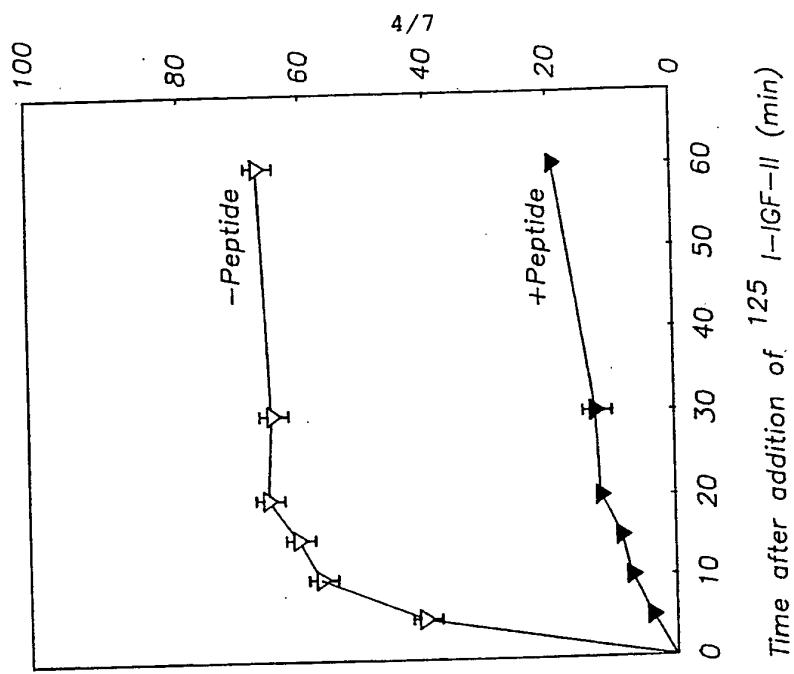


FIG. - 4B

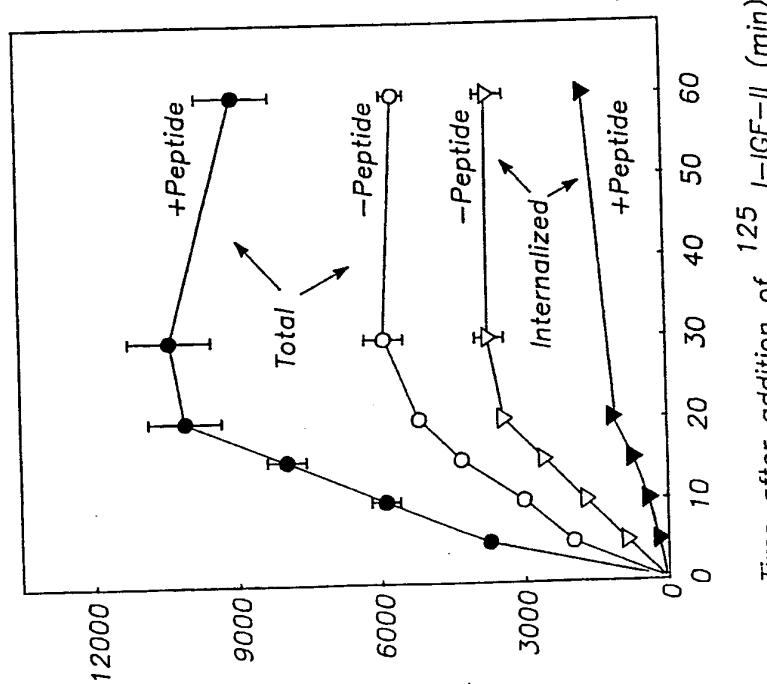


FIG. - 4A

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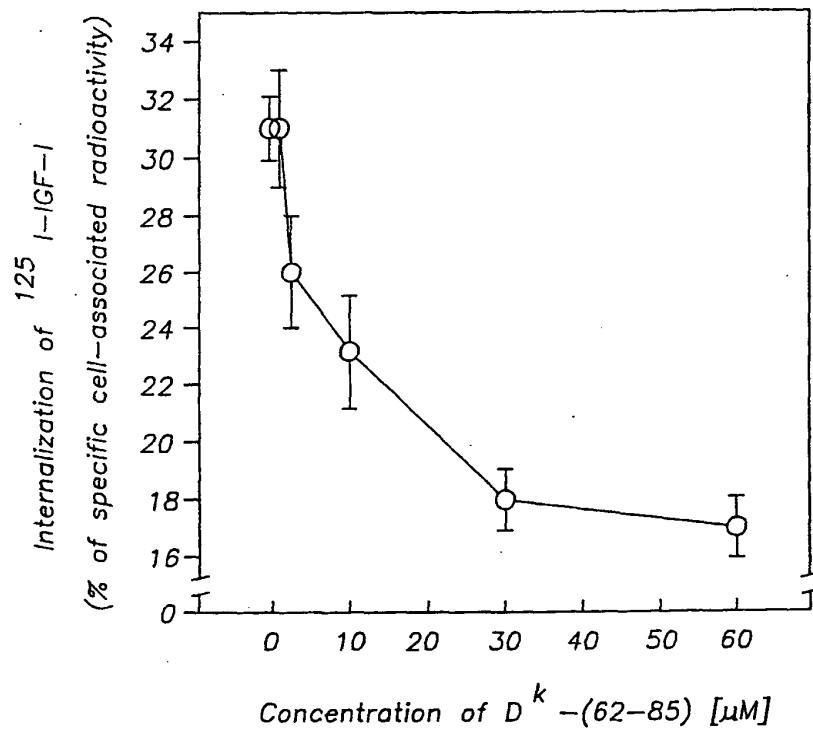


FIG. - 5

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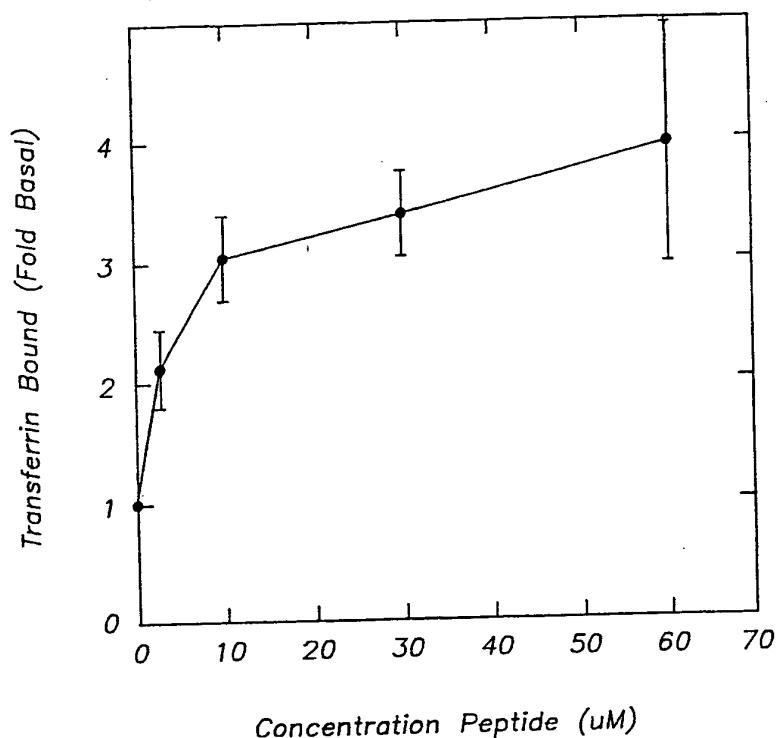


FIG. - 6

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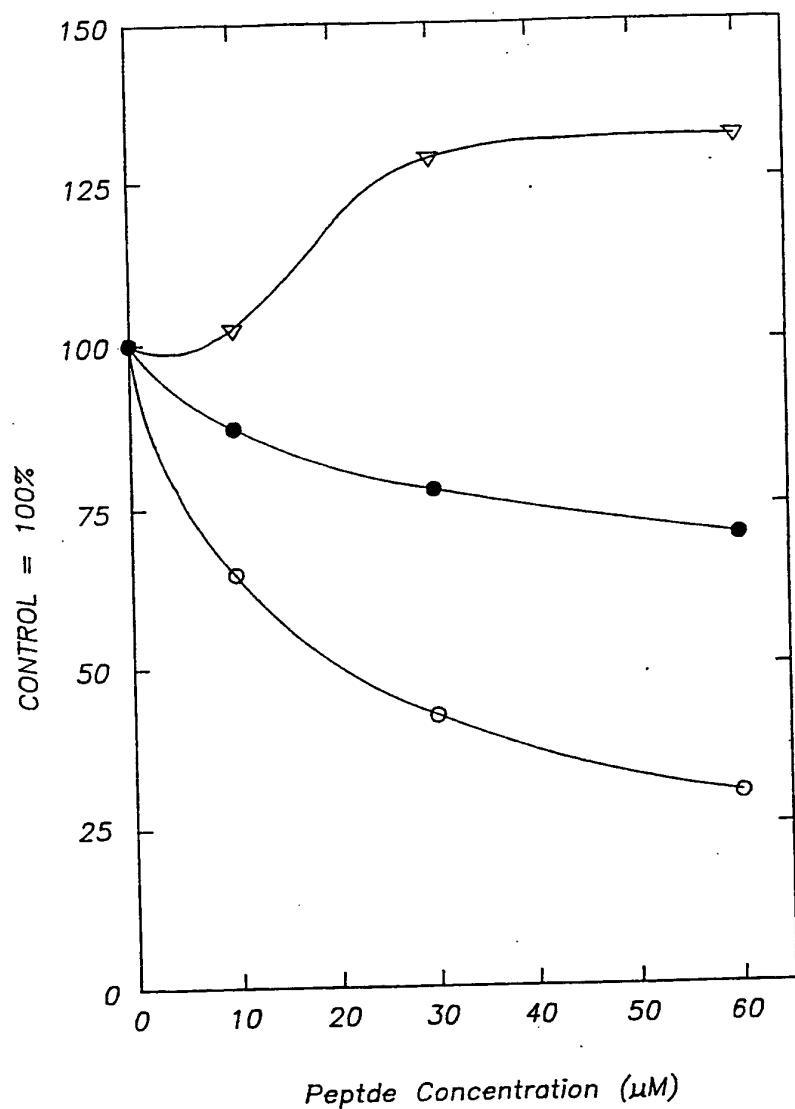


FIG. - 7

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US94/09189

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 38/00, 45/00; C07K 14/74  
 US CL : 530/300, 350, 395; 424/184.1; 514/2, 8

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/300, 350, 395; 424/184.1; 514/2, 8

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Automated Patent System and DIALOG (files 5, 155, 351, 399) Databases. Search terms: Major histocompat? insulin, EGF, GLUT?, glucose(w)transporter, surface(w)receptor.

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Journal of Biological Chemistry, Vol. 266, No. 20, issued 15 July 1991, J. Stagsted et al., "A preformed, ordered structure of a 25-residue peptide derived from a major histocompatibility complex class I antigen is required to affect insulin receptor function", pages 12844-47, see abstract.	1-86
Y	Cell, Vol. 62, issued 27 July 1990, J. Stagsted et al., "Regulation of insulin receptor functions by a peptide derived from a major histocompatibility complex class I antigen", pages 297-307, see abstract.	1-86

 Further documents are listed in the continuation of Box C. See patent family annex.

• Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E" earlier document published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search	Date of mailing of the international search report
26 NOVEMBER 1994	12 DEC 1994

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## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US94/09189

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Journal of Immunology, Vol. 143, No. 3, issued 01 August 1989, S. Verland et al., "Specific molecular interaction between the insulin receptor and a D product of MHC Class I", pages 945-951, see abstract.	1-86
T	Proc. Natl. Acad. Sci. USA, Vol. 91, issued September 1994, L. Olsson et al., "Regulation of receptor internalization by the major histocompatibility complex class I molecule", pages 9086-90, see abstract.	1-86